TABLE II. Total Recovery of Crystalline Vitamin B_{12} Added to Serum before Extraction. All values in $\mu\mu$g/ml.

Serum (Table I)	Endogenous Vit. B_{12} level	B_{12} added to serum before extraction	Expected additional B_{12} in extract*	Vit. B_{12} level assayed	% recovery of expected total
E	40	100	85	104	83
F	64	150	127	192	100
G	56	200	170	188	83
J	104	100	85	236	112
K	224	150	127	312	89
L	304	200	170	504	106
M	224	300	255	408	85

* Correction for 15% loss secondary to extraction procedure.

genous B_{12} level of the serum extract used in preparation of the standard curve. However, this error is minimized by choosing a serum with the lowest B_{12} level (least inhibition of binding of 100 $\mu\mu$g Co^{57}-B_{12} by I.F.).

Serum Vit. B_{12} concentrations in normal controls and in patients with pernicious anemia and leukemia reported above agree, in general, with levels obtained employing microbiological assay procedures(7,8). A more precise and critical comparison can be made only when many more sera are assayed simultaneously by both methods.

Summary. Based on some observed properties of the B_{12}-I.F. complex, an assay procedure is described for determining serum Vit. B_{12} levels using isotopically labeled Vit. B_{12} and intrinsic factor. 83%-112% of crystalline Vit. B_{12} added to serum was recovered in the assay. Concentrations of Vit. B_{12} in normal, B_{12} deficient, and leukemic sera were in the ranges, 104-328 $\mu\mu$g/ml, 0-72 $\mu\mu$g/ml and over 1000 $\mu\mu$g/ml respectively.

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Antigen Elimination from the Site of Arthus Phenomenon in the Guinea Pig* (26841)

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Almost 60 years have passed since Arthus (1), using horse serum injected into rabbits, described the phenomenon that bears his name, but its effect on the removal of antigen from the injection site has not been clearly delineated. The gross physical manifestations of this reaction are usually apparent 60 minutes after injection of the antigen, and thus one might anticipate an effect on antigen removal during the first few hours after in-

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jection. We have not found data in the literature comparing rate of removal of soluble antigen during the first 12 hours after subcutaneous injection into immune and control animals. Wadsworth(2) in 1904, showed that when virulent pneumococci were placed in the lungs of immune rabbits, the degree of septicemia was less than in controls. Immune animals would develop diffuse exudative lesions comparable to the lobar pneumonia of man, but they ordinarily survived, whereas control animals died with bacteremia.

Opie(3) demonstrated that more egg albumin remained at injection site at 24-72 hr in immune rabbits than in control animals. He did not make observations at less than 24 hours and used large amounts of antigen (25 mg).

Krause(4), studying tuberculous infection in the guinea pig, observed that in the immune animals, after virulent subcutaneous infection a great delay occurs in transmission of tubercle bacilli from the point of entry to the customary places of localization within the body; he attributed this delay to the mechanical effect of the inflammatory process.

Menkin's(5) studies showing slower removal of india ink or bacteria from the nonspecifically inflamed peritoneal cavity are not necessarily analogous to the effects of inflammation due to immune processes.

Rich(6), demonstrated that pneumococci are localized at site of intracutaneous injection in immune animals. He interpreted his data as showing that antibodies acted specifically to immobilize bacteria before the inflammatory response was apparent.

Recently, Talmage, Dixon, Bukantz and Dammin(7), and Law and Wright(8), studying the rate of antigen elimination after intravenous injection, noted a more rapid fall in blood antigen concentration in immune animals compared to normals.

In the following experiments, with the aid of radioisotopes, we have studied the effect of the Arthus phenomenon on elimination of the antigen from the subcutaneous tissue of immune and control guinea pigs. We have also observed the effect of such an immune response on rate of disappearance of non-antigen from the site of the Arthus phenomenon.

Materials and methods. A. Materials: 1. Young adult guinea pigs (280-300 g, about 4 weeks old) were fed Purina chow *ad libitum*. Immune and control animal pairs carry the same number for identification. 2. Armour crystallized bovine serum albumin, (BSA). 3. Human gamma-globulin (HGG), isolated from a myeloma patient(9). 4. Cyanocobalamin with Co^{60} incorporated biosynthetically ($B_{12}Co^{60}$). 5. Human plasma albumin (HPA). 6. Freund's adjuvant (Bayol 8.5, Arlacel 1.5, and 5 mg/ml of mycobacterium bovis). *B. Methods*: 1. For immunization each guinea pig received an emulsion of 2.5 mg crystallized bovine serum albumin in 0.25 ml saline and 0.25 ml Freund's adjuvant. Three weeks later sera from heart blood were tested qualitatively for precipitating antibodies; the test was positive in 22 out of the 26 immunized guinea pigs. *This was the only immunizing antigen* injected and throughout this report "immune animal" refers to one given BSA in Freund's adjuvant 3-5 weeks before these studies. 2. BSA and HGG were radioiodinated according to the technic of Talmage *et al.*(7); at least 95% of I^{131} was in trichloracetic acid precipitate. 3) The radioactive solution to be studied (0.1 ml) was injected from a tuberculin syringe into the pad of a rear foot. *Specific details concerning amount and kind of material injected for study are found in the legends for tables and figures.* 4) Radioactivity remaining in the injected foot was assayed *in vitro* or *in vivo* with a scintillation detector coupled to a single channel pulse height analyzer. When 2 different isotopes (I^{131} and Co^{60}) were injected simultaneously the amount of each remaining was measured by scintillation spectrometry.

Results. Swelling, redness and edema and increased heat were noted during the first hour in the antigen injected foot of immune animals having precipitins in their serum. The Arthus reaction was lacking or minimal in those guinea pigs which did not develop precipitins. I. Removal of antigen from the site of injection in immune and control guinea pigs: a) Fig. 1, 2 and 3 show radioac-

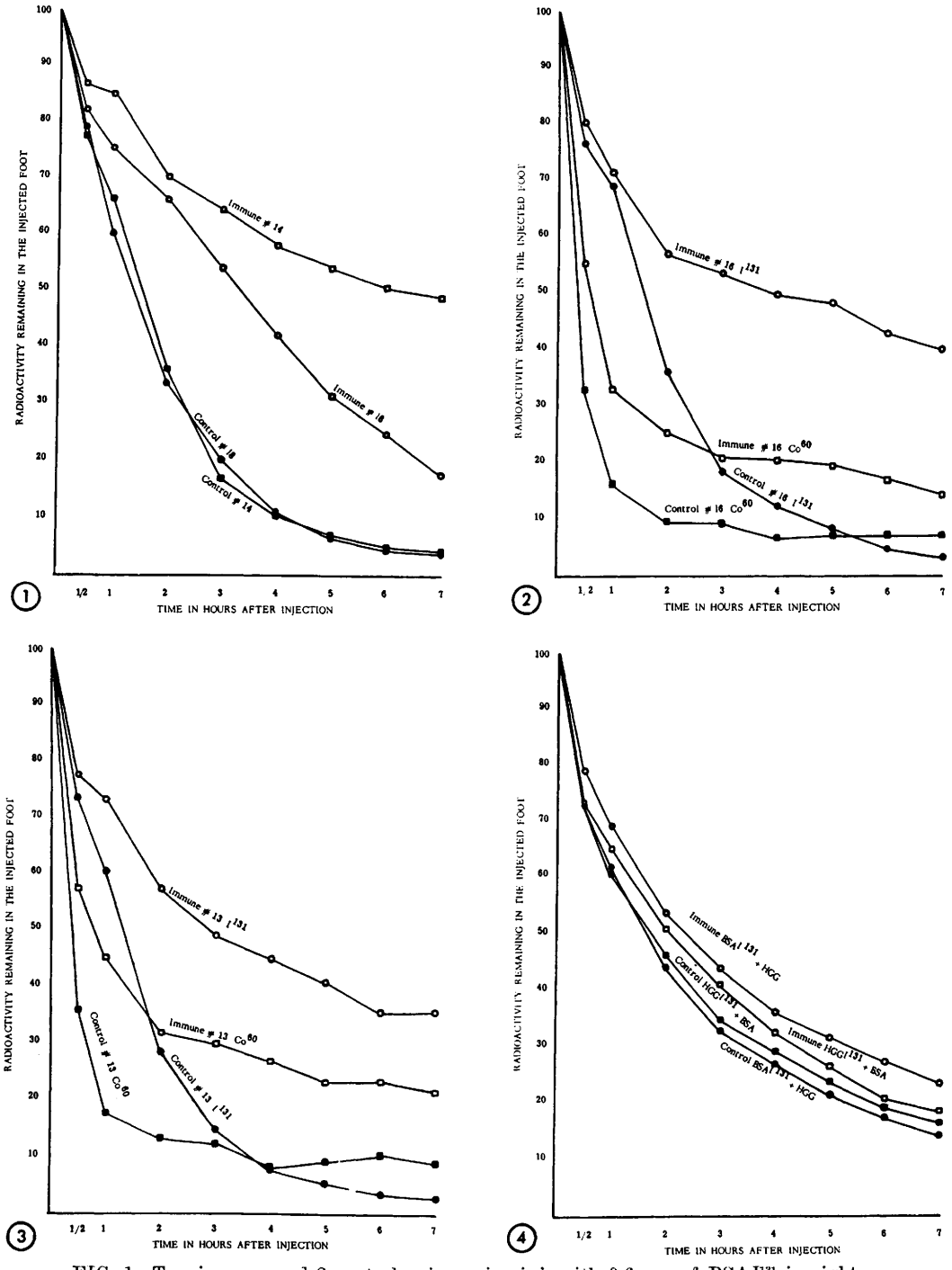

FIG. 1. Two immune and 2 control guinea pigs inj. with 0.6 mg of BSA I^{131} in right rear foot. Each line represents *in vivo* determination of radioactivity remaining in inj. foot in one animal.

FIG. 2. One immune and one control guinea pig inj. in right rear foot with a mixture of 0.6 mg of BSA I^{131} and 0.5 μg of Vit. $B_{12}Co^{60}$, the latter being in excess of B_{12} binding capacity of the protein. Each line represents *in vivo* determination of radioactivity due to I^{131} or Co^{60} remain-

TABLE I. Comparison of Amount of Antigen Remaining at Injection Site in Immune and Control Animals.

Animal pair No.	Hr after inj.	Right hind foot inj. with	Radioactivity (I^{131}) remaining in right hind foot (% of inj. dose) Immune guinea pigs	Control guinea pigs
6	.5	$BSAI^{131}$	84	42
7	.5	" + $B_{12}Co^{60}$	82	75
3	1	"	92	31
8	1	" + $B_{12}Co^{60}$	69	77
4	2	"	51	58
9	2	" + $B_{12}Co^{60}$	53	75
5	4	"	32	15
10	4	" + $B_{12}Co^{60}$	28	19
18	7	"	20	2
14	7	"	66	4
13	7	" + $B_{12}Co^{60}$	48	3
16	7	" + $B_{12}Co^{60}$	52	4
15	7	" + HPA	34	8
17	7	" + HPA	22	3
19	7	" + HGG	36	12
20*	7	" + HGG	25	14
21*	7	" + HGG	14	16
22*	7	" + HGG	20	19
23*	7	" + HGG	28	14
24	7	" + HGG	17	14
25	7	" + HGG	22	8
26	7	" + HGG	31	9
27	7	" + HGG	20	12
11	8	" + $B_{12}Co^{60}$	19	5
1	24	"	20	1
12	24	" + $B_{12}Co^{60}$	2	1

Radioactive antigen remaining in inj. foot of all immune and control guinea pigs. After sacrifice of animal right hind foot was amputated and radioactivity determined in a well scintillation counter. Animals inj. in right foot with $BSAI^{131}$ + HPA or $BSAI^{131}$ + HGG were inj. in left foot with $HPAI^{131}$ + BSA or $HGGI^{131}$ + BSA respectively. Dose of BSA, HPA and HGG = 0.6 mg of each in each inj. foot. Dose of $B_{12}Co^{60}$ = 0.05 μg.

* Immune animals with negative precipitin test.

tivity (as determined by *in vivo* counting) remaining in the foot at intervals up to 7 hr after injection of radioactive antigen. They demonstrate a marked slowing of removal of the antigen from the foot of the immune animals. The same trend is seen in Fig. 4 which shows mean radioactivity due to antigen in the injected foot of 9 immune and 9 control guinea pigs injected with a mixture of BSA and HGG.

b) Table I records the remaining radioactivity of $BSA^{I^{131}}$ counted *in vitro* in the injected foot of all of animals used in our experiments. Here, there is also a significantly greater retention of antigen in the foot of the immune animals. The discrepancy seen in 4 control animals, No. 8, 4, 9, 21, probably represents variable tissue sites of injection. The results of these *in vitro* radioactivity determinations are analogous to those observed *in vivo* before sacrificing the animals. We conclude that antigen disappears from the site of subcutaneous injection at a slower rate in the immune than in the non-immune guinea pig.

II. Effect of Arthus phenomenon on disappearance of non-antigen from injection site: a) Effect on the water soluble small molecular weight compound (Vit. $B_{12}Co^{60}$). Fig. 2 and 3 and Table II show that in the presence of an Arthus phenomenon there is a slowing in absorption of Vit. $B_{12}Co^{60}$ but this effect is not noted during the first hour after

TABLE II. Comparison of Amount of Non-antigen (Vit. $B_{12}Co^{60}$) Remaining in Control Foot and Foot with Arthus Phenomenon.

Animal pair No.	Hr after inj.	Right hind foot inj. with	Radioactivity (Co^{60}) remaining in right hind foot (% of inj. dose) Immune guinea pigs	Control guinea pigs
7	.5	$BSAI^{131}$ + $B_{12}Co^{60}$	49	55
8	1	*Idem*	48	51
9	2	"	31	25
10	4	"	18	8
16	7	"	13	2
13	7	"	22	5
11	8	"	7	3
12	24	"	14	9

Radioactivity of Vit. $B_{12}CO^{60}$ remaining in inj. foot. The difference between immune and control values for animal pairs #9, 10, 16, 13, 11, and 12 is significant ($P < 0.01$). Dose of BSA = 0.6 mg; $B_{12}Co^{60}$ = 0.05 μg. Radioactivity measured in well scintillation detector after sacrificing guinea pig and amputating foot.

ing in inj. foot.

FIG. 3. As for Fig. 2.

FIG. 4. Nine immune and 9 control guinea pigs inj. with $BSAI^{131}$ + HGG in right hind foot and $HGGI^{131}$ + BSA in left. Each line shows the mean of *in vivo* determination of radioactivity remaining in inj. foot. Dose of BSA and HGG = 0.6 mg of each in each inj. foot.

TABLE III. Comparison of Amount of Antigen and Non-antigen Protein Remaining at Injection Site in Immune and Control Animals.

Animal pair No.	Right hind foot (inj. with BSA I^{131} + HGG)		Left hind foot (inj. with HGG I^{131} + BSA)	
	Immune	Control	Immune	Control
19	36	12	25	14
20	25	14	24	15
21	14	16	10	17
22	20	19	14	21
23	28	14	15	12
24	17	14	20	13
25	22	8	16	12
26	31	9	12	12
27	20	12	13	18
Mean	24	13	17	15

In vitro determination of radioactivity remaining (% of inj. dose) in foot of 9 immune and 9 control guinea pigs sacrificed at 7 hr. T test indicates a significant difference ($P = <0.01$) between radioactivity remaining in right hind foot of immune and control animals. Dose of BSA = 0.6 mg; HGG = 0.6 mg in each rear foot.

injection of a mixture of antigen + $B_{12}Co^{60}$. Fig. 2 and 3 show the *in vivo* radioactivity depicting rate of removal of Vit. $B_{12}Co^{60}$ from injection site in 2 immune animals and 2 controls. Table II shows the remaining radioactivity of Vit. $B_{12}Co^{60}$ counted *in vitro* at time of sacrifice.

b) The effect of the Arthus phenomenon seen on the water-soluble small molecular weight compound was not noticed on the HGG^{I131} during a similar period of time. From Table III and Fig. 4 it can be seen that there is a very minimal difference in rate of elimination of HGG^{I131} in immune and control animals when it is injected along with BSA.

III. Effect of HGG on disappearance of BSA^{I131} from injection site in non-immune guinea pigs:

From Table I one might deduce that addition of HGG to the BSA^{I131} solution delayed the absorption of the latter from the injection site in non-immune animals. Control guinea pigs 18 and 14, injected only with BSA^{I131}, showed 2% and 4% of the injected BSA^{I131} remaining at 7 hours, whereas control guinea pigs No. 19 through 27, injected with a mixture of BSA^{I131} + HGG showed a much higher percentage of radioactivity remaining in the foot. However, when this was specifically tested in 4 additional non-immune guinea pigs, no effect of HGG in removal of BSA was discernible (Table IV).

Discussion. Lewis(10), Field and Drinker (11), and Barnes and Trueta(12) have demonstrated that substances with small molecular weight are absorbed more rapidly from the subcutaneous tissue than high molecular weight substances. We have noted the same results: When control guinea pigs were injected with a mixture of $B_{12}Co^{60}$ and BSA^{I131}, Vit. B_{12} left the injection site more rapidly than BSA (Fig. 2, 3).

When the same mixture was injected into immune animals there was a delay in removal of $B_{12}Co^{60}$ compared to that in the control (Table III, Fig. 2, 3), and removal of the antigen (BSA^{I131}) was much delayed compared to controls. It is apparent that the delay in elimination of $B_{12}Co^{60}$ from the injection site in the immune animals was due to the non-specific mechanical effect of the immune inflammatory process. On teleological considerations it would appear advantageous to have small molecular weight non-antigen materials (*e.g.*, toxins) held at the site of an Arthus reaction. It is likely that the delay in elimination of the antigen was primarily due to the antibodies but was contributed to by the non-specific effect of inflammation. Rich and McKee(13), depriving immune animals of their leukocytes, have pointed out that these animals were able to resist septicemia, at least temporarily, in the absence of any local inflammatory process, when pneumococci were injected intradermally.

Opie(14), has suggested that the specific inflammation at injection site is the result of

TABLE IV. Amount of BSA^{I131} Remaining at 7 Hr at Injection Site of Non-immune Guinea Pigs.

Animal No.	Right hind foot inj. with BSA^{I131}	Left hind foot inj. with BSA^{I131} + HGG
28	9	8
29	8	7
30	6	5
31	8	7

Radioactivity remaining (% of inj. dose) in foot of 4 non-immune guinea pigs inj. with 0.5 mg of BSA^{I131} in right foot, and 0.5 mg of a mixture of BSA^{I131} + HGG, in equal amounts, in the left.

the meeting of antigen and antibody in the tissues. The appearance of this inflammatory process probably further delays antigen elimination.

In our experiments, when serum was tested for radioactivity due to subcutaneously injected antigen, it was found that radioactivity in the serum of immune animals was always less than that of the control. This finding might have been anticipated from reports (7,8) demonstrating an accelerated rate of disappearance of intravenously injected antigen in immune animals. Data reported above show that less subcutaneous antigen reaches the blood stream in immune animals during the first 24 hours after injection.

Summary. An Arthus phenomenon delays removal of the antigen from the area. This is believed to be primarily due to the presence of antibodies. However, a non-specific effect due to inflammation must also be contributory to this slower removal of antigen because removal of a non-antigen from the Arthus reaction area is decreased.

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Prostatic Fraction of Acid Phosphatase in Human Serum.* (26842)

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There is evidence for non-identity of acid phosphatase from different tissues of the same species(1). Heterogeneity of this enzyme has been observed also within the same tissue where it may exist in several distinct forms(2,3). In serum, the level of enzyme activity represents the sum of several components, the characterization of which may be of diagnostic interest. Thus, the Fishman-Lerner method to measure that part of the activity in human serum which derives from the prostate gland has proved very useful in diagnosis of prostatic cancer. In fact, many clinical investigations demonstrate that most of the acid phosphatase of serum, which can be inhibited by L-tartrate, originates from the prostate gland(4,5,6). In this paper it will be shown that the tartrate sensitive fraction of acid phosphatase can be separated from the non-sensitive one by chromatography of serum proteins on anion exchange cellulose columns, and that the tartrate sensitive fraction has chromatographic properties similar to the enzyme extracted from prostate tissue.

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Materials and methods. Three samples of normal serum from young healthy males, 4 samples of serum from patients with benign