Elimination of I^{131} Labelled Homologous and Heterologous Serum Proteins From Blood of Various Species.*† (22930)

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The tracing of I^{131} labelled proteins has been used extensively to determine the rates of elimination or half-lives of serum proteins in various species. This technic has proven to be reliable, giving data comparable to those obtained with passively transferred, internally labelled proteins(1-3) and proteins traced by immunochemical determinants(4-5). Dixon and co-workers using the I^{131} technic to study the half-lives of homologous globulins(6) and albumins(7) in the circulation have shown 1) that the half-lives of homologous serum proteins vary widely from species to species and 2) that these half-lives can be correlated with the metabolic rate of the host. On the basis of observations in the rabbit it was believed that rates of elimination of homologous serum proteins and rates of non-immune elimination of heterologous serum proteins, prior to appearance of antibody were similar, suggesting that the metabolic characteristics of the host determined the rates of all non-immune protein catabolism(8). However, the present observations in other species indicate that this situation does not hold generally.

Materials and methods. The serum proteins used in this study are listed in the accompanying table. These proteins were trace labelled with I^{131} (I*) according to the method of Talmage *et al.*(9). Final I* protein preparations contained less than one atom of iodine per molecule of protein and 99% of the radioactivity was protein bound as determined by trichloracetic acid precipitation. The following animals were used: 72 guinea pigs weighing approximately 500 g, 19 albino rats weighing between 170 and 200 g and 75 rabbits weighing between 2 and 2.5 kg. The guinea pigs and rats were injected by intracardiac puncture and through the tail veins respectively, with 7.5 mg protein/kilo body weight, whereas the rabbits were injected in the ear veins with between 15 and 30 mg of protein/kilo body weight. The animals were bled at 1-6 day intervals beginning 2 or more days after the injection. Approximately 0.5 ml of guinea pig or rat blood was collected in tubes containing potassium oxalate and heparin. Three tenths ml aliquots of the whole blood were counted in a well type gamma counter and the counts converted to percent of injected I* protein remaining in the total blood volume, using an arbitrary correction factor for blood volume of 8% of the body weight. Rabbits were bled from the ear veins and the counts in the sera converted to percent of injected I* protein remaining in the total blood volume. Half-lives of the rates of disappearance of I* protein from the blood were obtained by graphic analyses. These analyses were based on the exponential elimination of the proteins which followed equilibration between intravascular and extravascular fluid spaces. To determine the effect, if any, of amount of serum protein injected on the half-life, guinea pigs and rabbits were injected with varying amounts of I*BSA or I*BGG and the half-lives were determined. Four groups of 4 guinea pigs each were injected with either 0.5, 7.5, 25 or 100 mg I*BSA/kilo body weight. Two groups of rabbits (21 animals in each group) were injected with I*BGG. One received 1 mg of the protein/rabbit and the other 100 mg/rabbit.

Results. The half-lives of all homologous and heterologous proteins used in this study plus some half-lives obtained from the observations of others are given in the accompanying table. In the guinea pig homologous globulin half-life was twice that of homolo-

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TABLE I. Half-Lives of Homologous and Heterologous Serum Proteins.

Animal		Protein	Investigator	Method of fractionation	Avg half-life in days
Guinea pig	A	GPG (P)	Author	Ethanol	5.4 ± .3‡
		GPGG	Dixon *et al.*(6)	Ammonium sulfate	5.4 ± .9
		GPSA (P)	Author	Ethanol	2.8 ± .2
		GPSA	"	Ammonium sulfate	2.6 ± .2
	B	RGG (P)	"	Ethanol	5.1 ± .2
		RGG (A)	"	"	4.8 ± .4
		RGG	Melcher & Masouredis(5)	Ammonium sulfate	4.5 – 4.7
		BGG (A)	Author	Ethanol	1.8 ± .1
		HGG (L)	"	"	1.9 ± .2
	C	RSA (P)	"	"	2.3 ± .1
		RSA (A)	"	"	2.2 ± .1
		BSA (P)	"	"	2.3 ± .1
Rat	A	Rat SA	Feldman*	Ammonium sulfate	1.9 – 2.2
		Rat GG	Author	*Idem*	5.5 ± .6
	B	RGG (P)	"	Ethanol	6.7 ± .7
		BGG	Pruzansky & Axelrod†	"	3.0
	C	RSA (P)	Author	"	1.1 ± .1
		BSA (P)	"	"	1.3 ± .1
Rabbit	A	RGG (A)	Dixon *et al.*(6)	"	4.6 ± .8
		RGG	*Idem*	Ammonium sulfate	5.7 ±1.2
		RSA (A)	Dixon, Maurer & Deichmiller(7)	Ethanol	5.7 ± .3
	B	BGG (A)	Author	"	2.2 ± .3
		HGG (L)	"	"	3.0 ± .4
		GPG (P)	"	"	1.6 ± .3
	C	HSA (A)	"	"	4.1 ± .4
		BSA (A)	"	"	4.3 ± .6
Mouse	A	Mouse GG	Dixon *et al.*(6)	Ammonium sulfate	1.9
		" SA	Dixon, Maurer & Deichmiller(7)	Electrophoresis	1.2
	B	RGG	Masouredis, Melcher & Shimkin(10)	Ammonium sulfate	5.3 ± .2
		RGG	Dixon, Maurer & Weigle(11)	*Idem*	5.1
		BGG (A)	Deichmiller*	Ethanol	1.5
	C	HSA (L)	Melcher, Masouredis & Shimkin(12)	"	1.5 ± .1

BSA = bovine serum albumin, BGG = bovine gamma globulin, HSA = human serum albumin, HGG = human gamma globulin, RSA = rabbit serum albumin, RGG = rabbit gamma globulin, GPSA = guinea pig serum albumin, GPGG = guinea pig gamma globulin, GPG = guinea pig globulin; (A) = Armour and Co., (P) = Pentex Incorp., (L) Lederle Laboratories.

* Unpublished data. † Personal communication. ‡ ± stand. dev.

gous albumin. Heterologous globulin half-lives varied considerably while those of heterologous albumins were similar. In the rat homologous globulin was eliminated much more slowly than homologous albumin. The half-life of RGG was longer and the half-life of BGG shorter than that of homologous globulin, while the heterologous albumin half-lives were shorter than that of homologous albumin. In the rabbits the half-life of homologous albumin was approximately the same as that of homologous globulin, the heterologous albumin half-lives were somewhat shorter than that of the homologous albumin, and the heterologous globulin half-lives were considerably shorter than that of homologous globulin. I*BSA was eliminated from the guinea pig at the same rate regardless of whether 0.5, 7.5, 25 or 100 mg of the protein were injected/kilo body weight. Also, the non-immune elimination of I*BGG in rabbit was the same regardless of whether 1 mg or 100 mg of I*BGG were injected.

Discussion. Three generalities can be made concerning the elimination of homologous and heterologous serum proteins from the blood of guinea pigs, rats and rabbits. First, there is no consistent relationship between the rates of elimination of homologous albumin and homologous globulin in various

species. In the guinea pig and rat as in the mouse homologous globulin half-lives are significantly longer than the albumin half-lives while in the rabbit, as in man, cow and dog the homologous albumin and globulin half-lives are similar(7). *Second,* no constant relationship exists between the rates at which homologous and heterologous gamma globulins are eliminated. In some instances heterologous gamma globulins are eliminated 2-3 times faster than the homologous gamma globulin and in other instances they are eliminated 2-3 times slower. On the other hand, the relative rates of homologous and heterologous albumin elimination are similar in all the species studied. In each species the half-life of homologous albumin is somewhat longer (20-50%) than the half-lives of heterologous albumins. *Third,* there is no consistent relationship between the rates at which various heterologous albumins or globulins are eliminated in a given species nor between the rates of elimination of a given protein in different species. The half-lives of different heterologous proteins may vary as much as 6-fold in a given species, and the half-lives of a single protein may differ more than 3-fold from one species to another.

The half-life of a protein in the circulation of a given species is probably dependent upon many factors among which are: the metabolic rate of the host, which correlates well with the half-lives of most homologous proteins, and the physical-chemical properties of the protein which would determine its physiological suitability (ability to circulate in the body fluids) and metabolic suitability (susceptibility to the host's enzymes) in the particular host. As appears to be the case, a given protein does not get similar physiological and metabolic acceptance in all heterologous species nor do different heterologous proteins in a given host. It is of interest that the physiological and metabolic suitability of a given heterologous serum protein in a particular species may have no relationship to its immunologic properties in that species. Proteins which appear most suitable physiologically may be extremely antigenic in the same species and vice versa(13).

RGG is unique in its ability to persist in the circulation of foreign hosts. Other heterologous proteins are eliminated from the guinea pig, rat and mouse 2-6 times as rapidly as RGG. In the mouse and rat RGG is eliminated even more slowly than are homologous serum proteins. RGG differs from the gamma globulin of other species not only in this *in vivo* behavior, but also in its N-terminal amino acid composition(14,15,16). Whether or not this chemical difference is related to the ability of RGG to persist in foreign species cannot be answered at the present time. In order to investigate the possibility that the *in vivo* behavior of RGG was the result of non-specific resistance to enzymatic hydrolysis, both I*RGG and I*BGG were incubated with various concentrations of trypsin and the percent of I* liberated from the protein was determined. Trypsin hydrolysed both of the proteins at approximately the same degree indicating that RGG possessed no particular resistance to *in vitro* hydrolysis by trypsin. Whatever the properties of RGG which enable it to circulate in foreign hosts they do not influence the behavior of other proteins in the same animal. When I*BSA and I*BGG were injected into guinea pigs, along with 1 ml of rabbit serum, the labelled proteins were eliminated at the same rates as when they were injected alone.

McFarlane(2) reported that the elimination of methanol fractionated homologous albumin, in the rabbit, was 30% faster than sodium sulfate precipitated albumin. Dixon *et al.*(6) also reported a shorter half-life in the rabbit for ethanol fractionated RGG than for ammonium sulfate precipitated RGG. This difference in rate of elimination between salt and alcohol fractionated serum proteins, however, is not evident in the guinea pig (table). The difference between the half-lives of alcohol and salt fractionated serum proteins, which appears to exist in the rabbit but not in the guinea pig, may be associated with either species difference or with technical factors in the preparation of the particular materials studied.

Summary. The rates at which heterologous serum proteins are eliminated from the blood

of guinea pigs, rabbits, rats and mice prior to the immune response may differ greatly from rates of elimination of homologous serum proteins. While elimination of homologous proteins, particularly albumins, appears to be related primarily to the metabolic rate of the host, elimination of heterologous proteins apparently reflects, in addition, the physiological and metabolic suitability of the protein to the particular host. Rabbit gamma globulin is unique in that it persists in foreign hosts as long or longer than do the homologous proteins. The half-lives of serum proteins in guinea pig are independent of the methods used for their separation (alcohol and ammonium sulfate fractionation).

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Viral Agents Oncolytic for Human Tumors in Heterologous Host. Oncolytic Effect of Coxsackie B Viruses.* (22931)

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Ability of many viral agents of low pathogenicity for man to proliferate in and destroy neoplastic human cells in tissue culture, has intensified the search for agents that produce such an effect *in vivo*(1-5). Several viruses produced oncolytic effects in animal tumors (6,7) and particular attention has been given to their effects on tumors of ascites and lymphoma types(8,9). The possibility of adapting a virus to multiply and later to become oncolytic in a tumor system which it initially failed to infect, has also been demonstrated (10,11). However, search for agents which are oncolytic for cancer in man thus far has made only very limited progress(12-14), possibly for want of adequate method of screening and assaying virus effects directly on human cancer cells *in vivo*. The high degree of cell and species specificity of most mammalian viruses makes it, *a priori*, unlikely that an animal tumor system will provide more than a model of specific virus-tumor relationships. Likewise, it is unwarranted to assume that behavior of a virus in tissue culture is necessarily a guide to its behavior on similar cells *in vivo*(8,15). However, a combination of the two approaches, transplantation of tissue culture grown human tumor cells into animals, might come closer to providing a practical method for *in vivo* study

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