Fate of Antibody Following *in vivo* Combination with Specific Antigen.* (20672)

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It is well known that the level of circulating antibody falls abruptly after the injection of specific antigen. This decrease in circulating antibody is presumably the result of the formation of antibody—antigen complexes which are quickly removed from the blood. The fate of the antibody following its combination with antigen and removal from the blood has not been described. In a previous study we traced the fate of an I^{131} labelled protein antigen during and following its *in vivo* combination with antibody(1). It was found that the antigen was catabolized rapidly following its elimination from the blood. The present report deals with the *in vivo* fate of the antibody portion of the immunologic complex.

We have labelled the gamma globulin fraction of rabbit antiserum to bovine albumin with I^{131} and traced the fate of the labelled antiserum after its passive administration into rabbits, some of which contained the specific antigen. I^{131} labelled proteins retain their radioactive label until they are degraded beyond the point of immunologic recognition, at which time the I^{131} appears in non-protein bound form, chiefly as iodide, and is excreted primarily by the kidneys(2). Therefore, the originally labelled homologous protein can be traced by determination of protein bound I^{131}, which exists in dynamic equilibrium in the intravascular and extravascular components of the plasma protein pool(3). The amount of protein catabolized can be determined from the measurement of non-protein bound I^{131} present in and excreted by the host.

Materials and methods. The gamma globulin fraction of pooled sera from rabbits repeatedly immunized with crystalline bovine serum albumin (BSA) (Armour and Co. lot No. 20) was obtained by ammonium sulfate precipitation(4). This gamma globulin fraction, containing 52 mg protein and 27 mg anti BSA antibody per ml, as measured by quantitative total and antibody nitrogen determinations(5), was labelled with I^{131} as previously described(6). The I^{131} labelled gamma globulin preparation (I*RGG) contained less than an iodine atom per molecule of protein, and approximately 99% of the I^{131} was protein bound as determined by trichloracetic acid precipitation. As has been previously reported, the degree of iodination of antibody molecules does not differ from that of other globulin molecules(7a,b,c). Therefore, it may be assumed that the I^{131}: protein ratio in the I*RGG was the same for both the specific anti BSA antibody molecules and the other globulin molecules. The anti BSA N : BSA N ratio at equivalence was approximately 5.5. Twelve young male albino rabbits weighing from 2.2 to 2.4 kg made up the 3 experimental groups used in this study. All were given non-radioactive iodide in their drinking water for several days prior to the injection of I*RGG in order to saturate iodine utilizing tissues and reduce non-specific retention of I^{131}. Rabbits were kept in individual metabolism cages for the collection of urine. Bladder urine was obtained by catheter and was combined with urine collected in the metabolism cage at each determination. The total amount of non-protein bound I^{131} in the body was calculated by multiplying the amount in 1 cc of whole blood by 37% of the body weight expressed in grams(1). Technics of protein bound I^{131} determination of plasma have been previously described(6).

*Group I: I*RGG.* Four rabbits each received intravenously a 2 cc injection of I*RGG containing 20 mg anti BSA antibody. Determinations of the protein bound I^{131} in the plasma and the non-protein bound I^{131} in total body and urine were made 1, 2, 3, 4, and 7 days after injection. *Group II: I*RGG followed by BSA.* Four rabbits each were in-

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jected intravenously with 4cc I*RGG containing 40 mg anti BSA antibody. Forty-eight hours later, immediately following determination of protein bound and non-protein bound I^{131}, they received an intravenous injection of 50 mg BSA. Protein bound I^{131} determinations of the plasma were made 1 hour, 6 hours, 1 day, 2 days, and 5 days after injection of BSA. Nonprotein bound I^{131} determinations of body and urine were made 6 hours, 1 day, 2 days, and 5 days after BSA. *Group III: BSA followed by I*RGG.* Four rabbits each were injected intravenously with 40 mg BSA and 24 hours later were injected intravenously with 5 cc I*RGG containing 50 mg anti BSA antibody. Protein bound I^{131} determinations of the plasma were made 1 hour, 6 hours, 1 day, 2 days, and 3 days after the I*RGG. Non-protein bound I^{131} in body and urine were determined 6 hours, 1 day, 2 days, 3 days, and 6 days after the I*RGG.

Results. The amount of I*RGG in total plasma volume of each rabbit throughout the period of observation is presented in Fig. 1. The total accumulative non-protein bound I^{131} found in urine and body for each rabbit is shown in Fig. 2.

*Group I: I*RGG.* In the animals receiving only antiserum, the I*RGG disappeared from the plasma rapidly during the first 2 days and then at a much slower rate for the remainder of the period of observation (Fig. 1). The initial rapid disappearance from the blood is caused in large part by equilibration of the labelled protein between the intra- and extravascular components of the plasma protein pool(8). The second, slower rate of elimination is the result of normal metabolic degradation of gamma globulin(9). The amount of non-protein bound I^{131} appearing during the first post injection day was greater than that appearing during subsequent days (Fig. 2). This difference was probably the result of rapid catabolism of any slightly altered protein and rapid excretion of the non-protein bound I^{131} in the I*RGG. The Group I rates of I*RGG elimination from the blood and its catabolism, as indicated by appearance of non-protein bound I^{131}, can be used as controls for comparison with the observations on Groups II and III, which received BSA in addition to I*RGG.

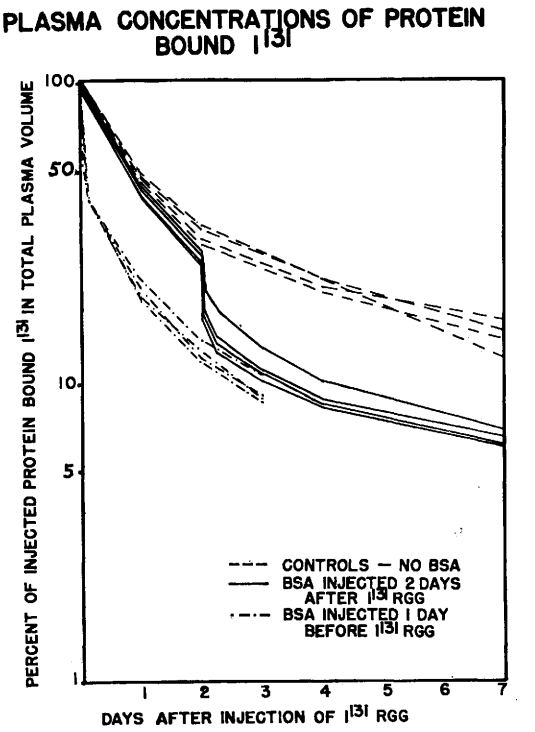

FIG. 1. % of injected I*RGG in total plasma vol. Individual line for each rabbit indicates values during course of observations. In Group II (solid lines) the administration of BSA on Day 2 is followed by a prompt drop in plasma I*RGG values to about one-half those seen in Group I controls and to the range of those of Group III.

*Group II: I*RGG followed by BSA.* This part of the experiment was designed to observe the effect of an excess of antigen on the fate of antibody which had equilibrated within the plasma protein pool, thereby having a distribution somewhat comparable to actively produced antibody. In these animals the blood levels of I*RGG and the amount of non-protein bound I^{131} two days after injection of I*RGG and immediately prior to the injection of BSA were similar to those found in the controls, as would be expected. However, within one hour after the injection of BSA, the I*RGG levels in the plasma fell from an average of 25% of the original injection to 17.7%, within 6 hours to 14.3 per cent, and within 24 hours to 11% (Fig. 1). Twenty-four hours after injection of BSA, the amount of I*RGG in the blood was approximately

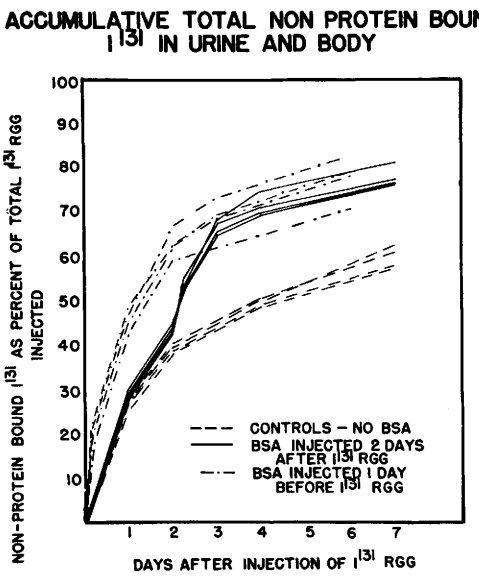

FIG. 2. Rate of appearance of non-protein bound I^{131} indicating catabolism of I*RGG. In Group II after injection of BSA the total non-protein bound I^{131} increases rapidly from values near those of the Group I controls to values comparable to those of Group III.

one-half of that expected without injection of BSA, according to Group I values. The rate of elimination of I*RGG from the blood during the one to 5 day period after injection of BSA closely paralleled the final rate of elimination in Group I. Coincident with the rapid loss of I*RGG from the plasma after injection of BSA, there was an increase in the rate of appearance of non-protein bound I^{131} (Fig. 2). During the first 6 hours after the BSA injection, almost 10% of the injected I^{131} appeared in the non-protein bound form in excess of the amount expected had no BSA been given, and during the first 24 hours this excess had increased from 15 to 20% of the original injection. The rate of appearance of non-protein-bound I^{131} fell off abruptly the second day following injection of BSA.

*Group III: BSA followed by I*RGG.* This group was injected to see whether there would be any difference in the elimination and catabolism of antibody when the order of antibody and antigen administration was reversed. As can be seen in Fig. 1, there was a rapid loss of I*RGG from the blood immediately after its injection in the presence of BSA. From one third to one fourth of the I*RGG disappeared from the plasma within 5 minutes, almost one half within one hour, 60% within 5 hours, and 80% within one day. The amount of I*RGG in the plasma one and 2 days after injection was slightly less than one half that found in other groups, indicating specific elimination of a little more than one half of the I*RGG as a result of the presence of BSA. Three days after injection of I*RGG, the plasma protein bound I^{131} levels in Group III were comparable to those in Group II one day after injection of BSA. Non-protein bound I^{131} rapidly appeared in this group after injection of I*RGG. Within one day 45% of the injected activity was detectable in the non-protein bound form. The amounts of non-protein bound I^{131} found in Groups II and III from the third day on, after both had received BSA, were similar.

Discussion. Since the gamma globulin preparation, I*RGG, was approximately one half specific anti BSA antibody it would be expected that only one half, *i.e.*, the antibody, would be affected by the presence of BSA. As demonstrated by Groups II and III in Fig. 1, approximately one half the I*RGG was rapidly eliminated from the circulation in the presence of an excess of BSA. In Group II, within one hour after the injection of BSA, 31% of the I*RGG estimated to be circulating had BSA not been given was eliminated from the circulation; within 6 hours, 43% was eliminated; and within one day, 50% was eliminated. It seems likely that the 50% of the I*RGG eliminated in the presence of BSA was the specific anti BSA antibody. Assuming that were so, the percentage of anti-BSA antibody eliminated would be twice the above figures for I*RGG elimination, since specific anti BSA antibody made up approximately one half the total I*RGG protein. Since the intravascular and extravascular plasma proteins are in dynamic equilibrium, not only would one half of the I*RGG be removed from the plasma, but actually one half of the I*RGG in the entire body would be removed from the body's protein pool. The elimination rate of the remaining, non-antibody, I*RGG in Group II 24 hours or more after BSA injection agreed well with the observed rates of gamma globulin catabolism

uncomplicated by the presence of antigen(9).

In Group III I*RGG underwent simultaneous equilibration and removal by virtue of the presence of BSA. Since the early rates of equilibration were not determined in Groups I and II, it is not possible to estimate the rate of I*RGG elimination caused by BSA in the first 24 hours in Group III. However, that the BSA induced elimination of I*RGG was completed within 24 hours is indicated by the fact that the one day plasma levels of I*RGG in Group III were slightly less than one half those in Groups I and II. It appears that the order of administration of antigen and antibody does not noticeably affect the rate of elimination of antibody from the circulation.

Associated with the elimination of anti BSA antibody from the circulation in the presence of BSA, there is an increase in the rate of appearance of non-protein bound I^{131} indicating an increase in the rate of catabolism of the I*RGG, presumably the anti BSA antibody. However, calculation of the exact rates of antibody catabolism is complicated by the fact that recovery of non-protein bound I^{131} was not complete. Since some non-protein bound I^{131} is eliminated in the breath and the feces and since collection of urine in metabolism cages is never complete, loss of some of the non-protein bound I^{131} is to be expected. The per cent of non-protein bound I^{131} lost can be determined by adding to the total protein bound I^{131} in the animal (approximately twice that in the plasma) the non-protein bound I^{131} present in and excreted by the host and subtracting the sum from 100%. Such calculations show the efficiency of our non-protein bound I^{131} collection for the 7-day period for Groups I and II and a 3-day period for Group III to be: Group I—87%, Group II—89%, and Group III—84%. There is little retention of non-protein bound I^{131} in pre-iodinated rabbits(8). If the observed Group II non-protein bound I^{131} values given on Fig. 2 are corrected for the 11% loss of excreted non-protein bound I^{131}, the increased accumulation of non-protein bound I^{131} expressed as per cent of anti BSA antibody theoretically present in the host had no BSA been given, was as follows: at 6 hours 39%, 24 hours 84%, and 48 hours 97%. These values, while admittedly only approximations, indicate that antibody catabolism is largely completed less than 24 hours after antibody is removed from the circulation as a result of the presence of circulating specific antigen.

If the non-protein bound values for Group III (Fig. 2) are corrected for the 16 per cent loss of non-protein bound I^{131}, the increased accumulation of non-protein bound I^{131} expressed as per cent of anti BSA antibody theoretically present in the host had no BSA been given, was as follows: at 24 hours, 67% and at 48 hours, approximately 100%. The observations on Group III are in general agreement with those of Group II and support the conclusion that the catabolism of antibody occurs rapidly after its elimination from the circulation.

The rapid rate of catabolism of antibody after its elimination from the circulation, presumably as part of an antigen—antibody complex or precipitate, is comparable to the rate of heterologous serum protein antigen catabolism under similar circumstances, as previously observed(1). This is in line with the observation that the rates of catabolism of homologous and heterologous serum proteins in the absence of an immune response are similar(8). It appears that the rabbit is equally capable of catabolizing foreign and native serum proteins and does not make a marked distinction between the two as far as rate of catabolism is concerned.

Pressman *et al.*, found that I^{131} labelled rabbit anti mouse kidney antibody, when localized in the glomeruli of mice, had a half-life of 20 to 21 days(10). The difference between the rate of loss of the antibody presumably fixed to the antigenic constituents of the glomeruli and the rate of degradation of antibody removed from the blood in the presence of circulating antigen might be in part related to the difference in species of host but more likely is the result of localization of the antibody. The antibody as part of a circulating antibody-antigen complex or precipitate would be taken up, in all likelihood, by reticuloendothelial cells which apparently have the ability to degrade protein rapidly.

The antibody fixed to the antigenic components of the glomeruli might not be exposed to cells with such proteolytic powers. If the antibody were attached to the glomerular basement membrane, which has been shown to be the most antigenic of the kidney's components(11), it might even be extracellular and therefore not exposed to proteolytic attack by intracellular enzymes.

Conclusions. In the presence of specific antigen 62% of the homologous anti-protein antibodies are rapidly removed from the circulation within one hour after injection and 86 per cent within 6 hours. Once removed from the blood, the antibodies are promptly catabolized, between one third and one half within 6 hours and virtually all between 24 and 48 hours. In the rabbit the rates of catabolism of anti protein antibodies and heterologous serum protein antigens after their elimination from the circulation as specific complexes are comparable.

Since the submission of this manuscript for publication a paper dealing with this subject by S. P. Masouredis, L. R. Melcher and M. B. Shimkin entitled "The Effect of Antigen Administration on the Behavior of I^{131} Labelled Rabbit Anti-Ovalbumin in Mice" has appeared in the *Journal of Immunology,* 1953, v71, 268.

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Reproducibility of X-Ray Survival Curves for Yeast Cells.* (20673)

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While investigating the influence of "modifying factors" (anoxia, protective chemicals, temperature, and physical state) on radiosensitivity of yeasts(1), it was found that the changes in radiosensitivity that had to be measured were relatively small. This made it necessary to develop a technic that would yield accurate and reproducible X-ray survival curves and to investigate cultural and physical conditions that might influence the radiosensitivity of the cells and the shape of their survival curves. These investigations are described in this paper. The influence of other modifying factors will be reported elsewhere.

Methods. Unless otherwise specified, a haploid strain of the yeast *Saccharomyces cerevisiae* was used; in one experiment a genetically related diploid strain was used, both strains being supplied by Prof. R. E.

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