Catabolism of I^{131} Labelled Bovine Gamma Globulin in Immune and Non-immune Rabbits.* (18996)

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In contrast to antigens such as pneumococcal polysaccharides(1,2), azo dye labelled proteins(3-6), rickettsia and viruses(7), which remain intact in the host's tissues for long periods of time, heterologous serum protein antigens appear to persist in the host's tissues for only a relatively short time(8,9). These heterologous serum proteins are not eliminated as such in appreciable amounts by the host but rather are catabolized. It has already been noted that this antigen catabolism is more rapid in immune animals than in non-immune (9).

The present study was undertaken to learn more about the catabolism of heterologous serum protein antigens with particular reference to the role played by specific antibody. We measured the rate of catabolism of I^{131} labelled bovine gamma globulin† (I*BGG) in non-immunized, actively immunized and passively immunized rabbits. Catabolic rates in non-immunized and actively immunized animals were compared in order to amplify the previously reported observation of increased rate of antigen breakdown in immunized animals. Catabolic rates in actively and passively immunized animals were compared in an attempt to learn whether factors other than the presence of specific antibody were necessary for the increased rate of immune antigen catabolism.

The I^{131} antigen label used in these experiments is well suited to antigen catabolism studies. As previously described, I^{131} can be attached to protein antigens without detectably altering antigenic specificity(9). *In vivo* the I^{131} antigen appears to retain its label as long as it is immunologically active. As the I^{131} protein antigen is catabolized, there is liberation of non-protein bound I^{131}, largely iodide, with small amounts of organic bound forms, both of which are rapidly excreted in the urine(9). Therefore, following the injection of I^{131} labelled antigens, the non-protein bound I^{131} in the body at any time plus the non-protein bound I^{131} excreted up to that time would represent the amount of I^{131} antigen catabolized.

Materials and methods. Three groups of young male albino rabbits weighing from 1.9 to 2.2 kg were prepared as follows: Six rabbits were actively immunized by 2 intravenous injections of 100 mg of BGG 13 and 6 days before the experiment. Five rabbits were passively immunized by 3 injections of rabbit anti I*BGG antibody: 62 mg intramuscularly 24 hours before antigen injection, followed by 15 mg intravenously and 15 mg intramuscularly one hour before. The total amount of antibody passively administered to each rabbit was calculated to be approximately equal to the amount of antibody in the actively immunized rabbits. Six untreated rabbits were used as controls. All rabbits were pre-fed nonradioactive iodide to prevent uptake of liberated I^{131} by the thyroid. Antibody used in passive immunization was prepared by three injections of 75 mg BGG into donor rabbits, which were then bled out 9 days after

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† The bovine gamma globulin was prepared by Armour Laboratories, Chicago, Ill.

the last antigen injection. The concentrated antibody solution used for injection contained 3.1 mg anti I*BGG/cc, as determined by quantitative precipitation. The preparation of the test antigen, I*BGG, and the methods for protein bound and non-protein bound I^{131} determinations have been previously described(10).

Experimental procedure. The rabbits of all 3 groups were given intravenously 37 mg of I*BGG containing approximately 500 μc I^{131}. Protein bound and non-protein bound I^{131} determinations of blood and urine were made 1, 6, 24 and 48 hours after injection of antigen. The rabbits were kept in metabolism cages for urine collection, and their bladders were emptied by catheter at each stated time interval to insure accurate urine collection. The total body non-protein bound I^{131} was estimated by multiplying the amount found in one cc of whole blood by 37% of the body weight expressed in grams. This factor for estimating total body non-protein bound I^{131} was obtained by measuring the dilution of I^{131} in the blood stream following the intravenous injection of blood containing I*BGG breakdown residues into animals with both ureters ligated to prevent excretion of I^{131}.

Results. Both the urinary non-protein I^{131} and the total (urinary and body) non-protein I^{131} are represented for each group of rabbits in Fig. 1 to 3. The accumulative non-protein bound I^{131} values are plotted as percentages of original I*BGG injection against time in hours.

In Fig. 1 (non-immunized rabbits) the I*BGG catabolism with liberation of non-protein bound I^{131} is shown to be relatively slow: a total of 21% had been catabolized at 24 hours and 34% at 48 hours. The amount of non-protein I^{131} found in the urine was slightly less than one-half the total catabolized at the 6, 24, and 48-hour determinations.

Fig. 2 (actively immunized rabbits) shows a rapid liberation of non-protein I^{131} as seen at the 1- and 6-hour determinations. At 6 hours nearly 60% of the injected I*BGG had apparently been catabolized; at 24 hours, 70%; and at 48 hours, 82%. The renal elimination of non-protein I^{131} lagged for the first 24 hours, following which rapid excretion took place.

In Fig. 3 (passively immunized rabbits) the total and urinary non-protein I^{131} curves are practically identical to those for the actively immunized group. Both the rapid I^{131} liberation during the first 6 hours and the 24-hour delay in urinary excretion are apparent.

Discussion. It is evident that the catabolism of I*BGG in immunized animals is much more rapid than in non-immunized animals, especially within the first 6 hours following antigen injection. During this period approximately 60% is catabolized in immune animals and only 7% in non-immune.

Because the rate of I*BGG catabolism is almost identical in both actively and passively immunized rabbits, it appears that nothing

FIG. 1, 2, 3.

Liberated non-protein bound I^{131} plotted as % of I^{131} in original I^{131} BGG injection against time in hr. Dotted lines indicate non-protein I^{131} in urine and solid lines indicate total non-protein I^{131} in both urine and body. Light lines represent individual animals and heavy lines the group averages. FIG. 1—non-sensitized rabbits. Fig. 2—actively sensitized rabbits. Fig. 3—passively sensitized rabbits.

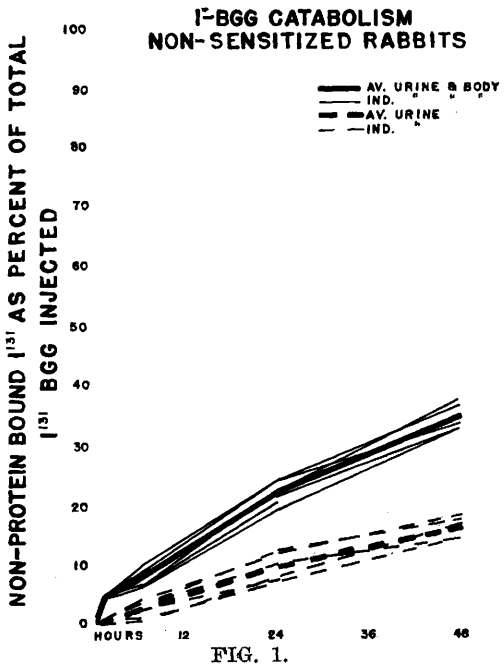

FIG. 1.

10. Talmage, D. W., Dixon, F. J., Bukantz, S. C., and Dammin, G. J., in press.

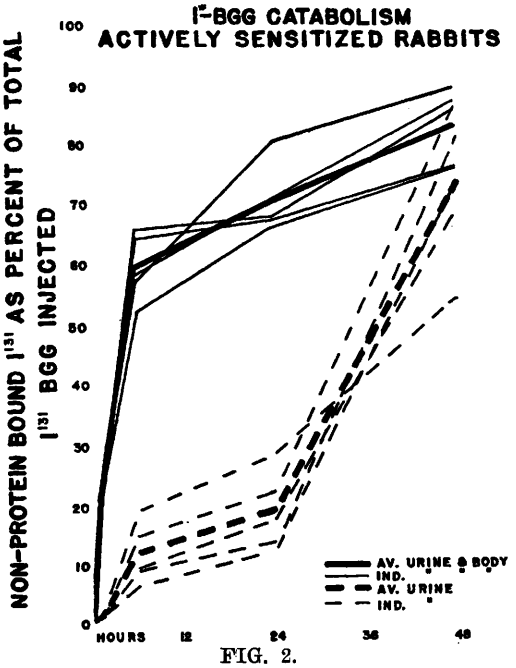

FIG. 2.

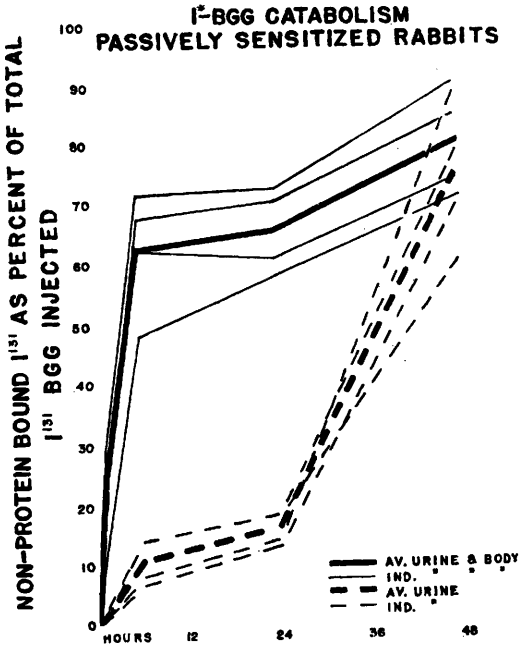

FIG. 3.

other than the presence of specific antibody is necessary for rapid immune antigen catabolism. Whether or not any cellular enzymatic adaptation which facilitates antigen catabolism occurs during the process of active immunization cannot yet be stated definitely. However, there is no evidence in this experiment of such a change.

The fact that only antibody is necessary to change slow non-immune antigen catabolism to rapid immune catabolism offers several clues to the mechanism of immune antigen catabolism. It seems most likely that the presence of antibody would result in antibody-antigen complexes which would be taken up by phagocytic cells. The phagocytic cells would in turn rapidly catabolize the antigen (and perhaps the entire complex) with liberation of non-protein I^{131}. This mechanism fits in with the observed slow non-immune antigen catabolism where, in the absence of specific antibody, no antigen complexes large enough to stimulate phagocytosis form.

As for the slow non-immune catabolism, it is most likely that the I*BGG enters the plasma protein pool, has free access to all tissues, and is utilized by the tissues, as is the native serum protein(11). It is of interest that the half-life of I*BGG in the non-immune rabbit as measured by liberation of non-protein I^{131} is about 3 days, which agrees well with the half-life arrived at by measurement of antigen disappearance from the blood(10).

Conclusions. 1. The catabolism of I*BGG in immune rabbits is much more rapid than in non-immune rabbits, especially within the first 6 hours. 2. Only specific antibody is necessary for the rapid immune catabolism, as indicated by the identical rates of I*BGG catabolism in actively and passively immunized rabbits.

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