Then we took up the syrup with absolute alcohol and treated it with about twice its volume of dry ether. The resulting precipitate was filtered and the filtrate was evaporated in a vacuum desiccator over phosphorus pentoxide and calcium chloride.

From the thick syrup after several days the 2-desoxy-D-ribose was obtained in crystalline form. This was washed with a small amount of cold *n*-propyl alcohol, mp 80°. The yield of the sugar was a little more than 0.3 g. The sugar gave a strongly positive Dische and Kiliani test. Its specific rotation was as follows:

$$[\alpha]_D^{22^\circ} = \frac{-1.13^\circ \times 100}{1 \times 2} = -56.5^\circ \text{ (in water)}.$$

Benzylphenylhydrazone.—0.3 g of 2-desoxy-D-ribose was dissolved in 1.5 ml of *n*-propyl alcohol, and 0.39 g of freshly distilled benzylphenylhydrazine was then added. After 3 hr in a desiccator over calcium chloride, the mixture had changed to a crystalline mass. It was washed with ether and recrystallized from *n*-propyl alcohol. Yield, 0.4 g. The substance melted at 129° (uncorrected).

$C_{18}H_{22}O_3N_2$ (314.2) Calc, N 8.92; found, N 8.94.

Its specific rotation was

$$[\alpha]_D^{22^\circ} = \frac{-0.33^\circ \times 100}{1 \times 2} = -16.5^\circ \text{ (in pyridine)}.$$

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The Effect of Sensitization and X-Radiation on the Metabolism of I^{131} Labeled Proteins[1]

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Various methods of tracing antigens in animal tissues have been employed since Metchnikoff's first titrations (1897) of tetanus toxin localized in mouse tissues (*1*). Among other nonradioactive agents that have been used as tracers are arsenic-proteins (*2*), iodinated serum (*3*), colored antigenic proteins prepared by azo conjugation (*4–7*), and fluorescent antibodies prepared by isocyanate conjugation (*8*). Since the introduction of radioactive isotopes into medical research, several such compounds have been so employed. Tobacco mosaic virus tagged with P^{32} has been traced in mice (*9*), I^{131}-tagged antibody has been traced in rats and mice (*10*), and I^{131}-tagged proteins have been traced in guinea pigs (*11*). The radioactive isotopes lend themselves extremely well to quantitative work and to studies of tissue localization by radioautography.

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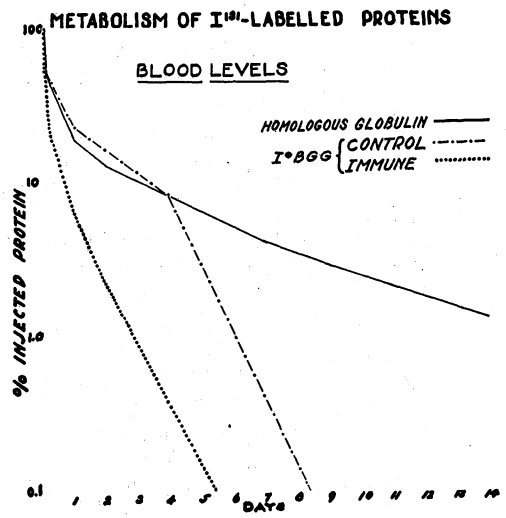

FIG. 1. Elimination curves for I*BGG in normal control and immune rabbits and homologous globulin (I*RGG) in normal control rabbits.

In particular the I^{131} label is desirable because:

1. It can be attached to proteins in traceable amounts without measurably altering their immunologic specificity.
2. Available evidence indicates that the I*[3] protein bond is a stable chemical link which *in vitro* resists wide changes in temperature and pH, salt exchange dialysis, prolonged storage and enzymatic action (*11, 12*). *In vivo* it appears that the iodine remains attached to the protein as long as the latter is immunologically detectable.
3. Prompt excretion of the I* label liberated by antigen metabolism can be accomplished by iodine prefeeding to saturate the iodine-utilizing tissues.
4. The I* liberated by protein metabolism is not appreciably incorporated either by synthesis or interchange into the rabbit's own proteins as determined by activity measurements of plasma protein fractions after administration of I* labeled homologous globulin. Furthermore, I* injected as inorganic iodide into iodine-prefed animals is rapidly excreted unchanged in the form of iodide. In contrast, the injection of I* attached as a protein label is followed by the excretion of nonprotein organic combinations of I*, probably diiodotyrosine and perhaps other amino acid forms, as well as iodide, suggesting actual protein degradation prior to liberation of the iodine label. This difference in excretion forms was determined by paper partition autoradiograms (*13*).

Labeled proteins were injected intravenously into 2-kg rabbits as follows: Labeled homologous globulin (I* RGG) to normal rabbits; labeled bovine γ globulin (I* BGG) to normal rabbits in amounts of 500, 75, or 1 mg; 75 mg I* BGG to rabbits immunized 20 days earlier by an intramuscular injection of 325 mg bovine γ globulin adsorbed on aluminum hyroxide; 75 mg I* BGG to rabbits, 48 hr following their exposure to 500–600r of 200 kv whole-body x-ray; 10 mg I* BGG to normal mice; and 90 mg I* BGG coupled with diazotized *p*-aminobenzoic acid (P-AP*) to normal rabbits.

All tracer studies were done by β-counts of dried

[3] I* hereinafter refers to I^{131}.

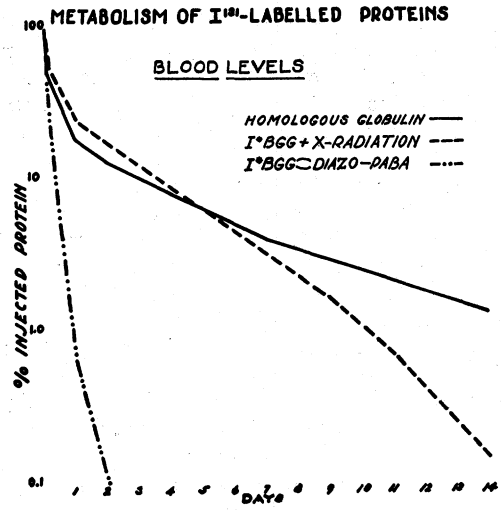

FIG. 2. Elimination curves for homologous globulin (I*RGG) and P-AP* (I*BGG-diazo-PABA) in normal control rabbits and I*BGG in x-radiated rabbits.

blood or plasma, homogenized tissue, urine, and protein-free supernates of blood and urine, with corrections for self-absorption of samples when necessary. Quantitative microprecipitin (*14, 15*) determinations of immunologically active protein in the blood agreed with protein-bound radioactivity. Tissue antigen concentrations were calculated by determining tissue hemoglobin and then subtracting from whole tissue activity that activity due to contained blood.

Blood levels. The rate of disappearance of labeled protein from the blood proved to be relatively constant and characteristic for each experimental group. The disappearance rate of I*RGG provides a base line for comparison with the other rates (Fig. 1). There is a rapid fall in blood activity during the first 24 hr after injection, at the end of which time approximately 20% of the original injection remains in the blood. This rapid drop within the first day is probably in part caused by mixing of the labeled protein with the extravascular, extracellular proteins, or "lymph proteins" (*16*). Equilibrium of labeled globulin in serum and lymph protein is apparently established by 24 hr. After this "dilution phase" there is a gradual straight line loss of labeled globulin from the circulation, which we have termed the "nonimmune elimination phase."

The blood disappearance curve for all doses of I* BGG (1–500 mg) in normal rabbits during the first 4 days closely resembles the curve with I* RGG. After 4 days, however, and just preceding the appearance of circulating antibody, the rate of antigen elimination from the blood becomes more rapid and continues so until the ninth day, when less than 0.1% of original injected protein can be found in the entire blood volume. This rapid third phase we have termed the "immune phase."

Immunized rabbits eliminate I* BGG from the blood much more rapidly than controls. There is apparently a simultaneous action of dilution and immune elimination during the first 24 hr, followed by immune elimination exactly paralleling that found in normal rabbits from fourth to ninth days. In immune rabbits, circulating antibody was present before injection of antigen. Less than 0.1% of the original injected protein could be found in the entire blood volume on the sixth day. Fig. 1 presents a comparison of disappearance curves for I* RGG in normal rabbits and I* BGG in normal and sensitized rabbits.

X-radiation apparently interfered with the development of an immune state, as shown in Fig. 2. The dilution and nonimmune elimination phases for the irradiated group are identical with those of the normal group for 4 days. In the irradiation group, however, an immune elimination rate fails to develop after the fourth day, the elimination rate resembling that of I* RGG for several additional days. The rate of disappearance gradually increases and approaches the immune rate. Circulating antibody was demonstrated in only a small proportion of the irradiated animals by the fourteenth day after injection.

P-AP* disappears from the circulation of normal rabbits at a rate more rapid than any of those previously described. In 2 days less than 0.1% is found in the entire blood volume (Fig. 2).

Urine excretion. Total urine collections were obtained in standard metabolism cages. The rabbits receiving I* BGG excrete, in the urine, a major portion of the injected activity in the form of radioactive iodide and smaller amounts of organic iodine combinations. None of the activity in urine is protein-bound, and antigen cannot be detected serologically. The rate of appearance of radioactivity in the urine reflects the rate of elimination from the blood. In the immunized group, 50% of the injected activity is found in the urine by the third day, in the control group by the fourth day, and the x-radiated group by the sixth day.

Normal mice injected with 10 mg of I* BGG excrete 70% of the injected dose of activity in the form of nonprotein-bound I* within 3 days.

I* excretion by rabbits given P-AP* is slower than after I* BGG injection. This slower excretion we found related to tissue retention of the P-AP*.

Tissues. When I* BGG is used as the antigen, there is no selective uptake or retention by any particular organ or tissue. The tissues vary somewhat in antigen content, but the levels of antigen concentration never exceed that of blood except in rabbits dying in anaphylaxis. The antigen content of blood and tissues diminishes at an almost equal rate, suggesting that the tissue content is dependent on the blood level. Estimated total carcass antigen values for normal rabbits on the ninth day and immunized rabbits on the sixth day are in the range of 0.3–0.5% of the original injection. Lung, liver, and heart yield slightly higher antigen levels than other organs. There is no apparent uptake or retention of antigen by mesenteric lymph nodes, spleen, or appendix.

In fatal anaphylaxis there is rapid loss of activity

from the blood and rapid accumulation in the lungs, occasionally to more than 8 times the concentration noted in the blood. Tissue autoradiographs reveal that the major portion of the antigen is concentrated in homogeneous, eosinophilic, intravascular masses. There is a similarly rapid but less marked accumulation in the liver, never exceeding 1.5 times the concentration in the blood.

P-AP* is rapidly lost from the circulation, 0.1% of injected protein remaining after 48 hr. In spite of this precipitous drop, the tissues studied (liver, spleen, lung, heart, kidney, appendix, and lymph node) contain significant amounts of radioactivity at 48 hr. Tissues highest in P-AP* content are the spleen, liver, and lymph nodes. Less marked retention is found in lungs and kidneys. At 10 days, the only tissues measured—spleen, liver, kidney, and lymph node—still retain radioactivity. Antigen retention is never observed when I* BGG is used, except in fatal anaphylaxis.

The data reported support the view that I* is a valid protein label *in vivo*. That is, after I* protein injection, protein-bound I* in the body fluids and tissues represents the originally labeled protein, whereas nonprotein-bound radioactivity indicates catabolism of the originally labeled protein. Characteristic curves of elimination of protein-bound activity from the blood are observed for each of the experimental groups described, and each shows a remarkable constancy. Specific phases, representing rates of elimination, can be identified as components of the curves. The manner of elimination of I* RGG from the rabbit's circulation resembles, in general, the curve of elimination obtained following injection of similar proteins labeled with C^{14} and N^{15} (*16*). Thus, I*-labeled proteins are handled like those proteins in which the radioactive label is more intimately incorporated into its molecular structure.

The I* BGG, irrespective of size of dose, leaves the blood stream of the normal rabbit rapidly, less than 0.1% of the injected dose being present at the ninth day. Antibody appears on the seventh day, rises, and persists after antigen is no longer detectable in the blood or tissues. The blood level of I* BGG in immunized rabbits drops more rapidly than in normal rabbits; in x-radiated, more slowly than in normal rabbits. The degree of active immunity determines the characteristics of the elimination curve.

Rapid disappearance of I* BGG from the blood is reflected in rapid appearance of I* as iodide and in organic but not protein combination in the urine. Further evidence that disruption of the protein molecule occurs is suggested by the observation that both organic and inorganic combination of I* are excreted. Normal mice given I* BGG behave essentially like normal rabbits with reference to the rapid excretion of nonprotein-bound activity in the urine.

No retention or selective localization of antigen in the tissues has been observed in rabbits given I* BGG. The fall in tissue content of antigen roughly parallels that of the blood. In immunized rabbits not dying in anaphylaxis there is no particular tissue localization, but the lungs, liver, and myocardium contain more activity per gram of blood-free tissue than do the spleen and appendix. In fatal anaphylaxis, antigen is rapidly lost from the blood and accumulates primarily intravascularly in the lungs.

In contrast with the above findings with I* BGG, we observed P-AP* to leave the blood more rapidly, less than 0.1% of the injected dose being present at 48 hr. Moreover, less activity appears in the urine and more is retained in the tissues, particularly the liver, spleen, and lymph nodes. The appendix, although a lymphoid tissue, does not respond in the same manner as the spleen and lymph nodes to foreign proteins labeled in this manner. Thus, P-AP*, though a soluble protein conjugate, is metabolized differently from lightly iodinated proteins, in that it is removed from the blood more rapidly and deposited in the reticuloendothelial system.

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