

To substantiate further the observation that the SPI is increased by estrogen, therapy was discontinued in 10 individuals and the course of the SPI followed for varying intervals thereafter. In all instances the SPI gradually fell toward the normal control range (Fig. 2). Although the data are limited it appeared that at least four weeks were required for maximal fall.

These findings suggest the possibility that the physiologic rise of the SPI of normal pregnancy is a phenomenon secondary to increased elaboration of estrogen since early and continued elaboration of large amounts of estrogen characterizes normal pregnancy. The data may also indicate that the tolerance for thyroid hormone is increased by estrogen. The clinical implications of these findings and the elucidation of the mechanisms involved in the response, remain to be evaluated.

**Summary.** The serum precipitable iodine (SPI), an index of the level of the circulating

thyroid hormone, rises in men or women during the administration of estrogen and falls to control values when the estrogen is discontinued.

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Received October 30, 1952. P.S.E.B.M., 1952, v81.

## Quantitative Study of Arthus Reaction and of Cutaneous Anaphylaxis Induced Passively in the Rat.\* (19949)

ZOLTAN OVARY† AND OTTO G. BIER

*From the Clinic of General Medicine and Therapeutics, University of Rome, Italy, and the Department of Immunology, Instituto Biológico, São Paulo, Brazil.*

The severity of the Arthus reaction in relation to the amount of antibody has been thoroughly studied by Fischel and Kabat in the rabbit(1), as well as by Benacerraf and Kabat in the guinea pig(2). No information is available, however, concerning the amounts of antibody required to induce the Arthus phenomenon in the rat. Kenton(3) was able to provoke direct Arthus reactions in the rat by the intraperitoneal injection of a large amount (5 ml) of rabbit antiovalbumin serum followed 3 hours later by the intracutaneous injection of the specific antigen. The anti-serum was assayed by the collodion particle

method and had a titer of 1:5000, but was not analysed for its antibody nitrogen content. The present investigation was undertaken to correlate the severity of passive direct or reverse Arthus reactions in the rat with the amounts of antibody required for their production. It also deals with the production of passive cutaneous anaphylaxis, as revealed by the intravenous injection of a mixture of antigen and a dye ("Geigy Blue 536") in animals previously sensitized by the intradermal injection of antibody. Circular blue spots develop at the sensitized sites as a consequence of the release of histamine which results from the interaction of antigen and cell-fixed antibody. As shown in this laboratory(4), the latter reaction is fundamentally different in mechanism from the Arthus phenomenon.

\* This investigation was conducted at the Dept. of Immunology, Instituto Biológico, São Paulo, Brazil, and aided in part by a grant from the National Research Council of the Brazilian government.

† Present address: Via Bufalini 8, Rome, Italy.

**Materials and methods.** Rabbit antiovalbumin and antipneumococcus serum type III were analyzed for antibody nitrogen by the quantitative precipitin method(5). Three times recrystallized egg albumin (Ea) and a highly purified preparation of SIII were used as antigens. The anti-Ea serum contained 0.307 mg and anti-SIII serum 1.593 mg AbN per ml.† Dilutions of the antigens or of the antisera to be injected intracutaneously were made in saline such that desired amounts were contained in a volume of 0.1 ml. For the intravenous injections the volume was 1 ml. Rats weighing  $100 \pm 20$  g were used throughout the experiment. The hair of the abdomen on the inoculation sites was simply clipped, no use being made of any depilatory or shaving in order to prevent nonspecific irritation of the skin. In performing the intradermal injections for the induction of passive cutaneous anaphylactic reactions care was taken not to pinch the skin and a sharp 26-gauge needle was first introduced through the skin and then had its direction reversed so as to deposit the inoculum properly in the dermal layers.

For the production of direct Arthus reactions antibody was given intravenously followed immediately thereafter by antigen intradermally. Vice versa, for the reverse Arthus reaction antigen was given intravenously and antibody intradermally. The rats were killed by hitting them on the head 4 hours later, their skin opened and reflected in order to observe the results on its inner surface. The intensity of the Arthus reactions was graded according to the diameter of the hemorrhagic area:  $\pm$  (less than 5 mm), + (5-10 mm) ++ (10-15 mm) +++ (15-20 mm) and ++++ (more than 20 mm). As to the passive anaphylactic reactions they were elicited by the intradermal injection of the antibody followed 4 hours later by the intravenous injection of a mixture of 0.5 ml of an adequate dilution of antigen plus 0.5 ml of 0.5% "Geigy Blue 536". The animals were

TABLE I. Severity of Reverse Arthus Reaction in the Rat in Relation to Amount of Rabbit Antiovalbumin.

Antiovalbumin N., $\mu$ g	Reactions			
60	++++	+++	+++	+++
30	++	++	++	++
20	++	++	++	++
10	+	+	$\pm$	$\pm$
5	$\pm$	$\pm$	0	0
1	0	0	0	0

Animals received 30 mg Ea intrav. followed immediately thereafter by the intradermal dose of antibody.

TABLE II. Relationship between Severity of Reverse Arthus Reaction in the Rat and Amount of Intradermally Injected Rabbit Anti-SIII.

Anti-SIII N., $\mu$ g	Reactions			
20	+++	++	++	++
10	++	++	+	
5	$\pm$	$\pm$	$\pm$	0

Animals received .25 mg SIII intrav. followed immediately thereafter by intradermal dose of antibody.

TABLE III. Passive Cutaneous Anaphylaxis in the Rat in Relation to Amount of Rabbit Antiovalbumin and of Rabbit Anti-SIII.

Antibody- N., $\mu$ g	Reactions				
(A) Anti-Ea					
2	++++*	+++			
1	++	++	$\pm$	$\pm$	$\pm$
.5	+	+	$\pm$	$\pm$	0
.25	0	0	0	0	0
(B) Anti-SIII					
2	+++	+++			
1	+	+	+		
.5	0	0	0		
.25	0	0	0		

Animals received 15 mg Ea or .15 mg SIII intrav. 4 hr after intradermal dose of antibody.

\* The results in the vertical columns correspond to dilutions of antibody tested on the same animal.

killed 10-15 min. after the injection of the antigen, the reactions examined and the sizes of the spots graded as described for the Arthus reactions.

**Results and discussion.** The results concerning the reverse Arthus reaction are summarized in Tables I and II. The data show that with both systems Ea-anti Ea and SIII-anti SIII the amount of antibody required for minimal Arthus reactions ( $\pm$  to +) is between 5 and 10  $\mu$ g AbN. This value

† We are indebted to Dr. Michael Heidelberger, of the College of Physicians and Surgeons, Columbia University, New York, for samples of type III antipneumococcal serum and type III polysaccharide (lot 202).

is of the same order of magnitude as the one found by Benacerraf and Kabat(2) for the reverse Arthus reaction with anti-Ea in guinea pigs of  $250 \pm 50$  g.

By intravenous sensitization with anti-SIII followed by the intradermal injection of 0.20 mg SIII, direct Arthus reactions of medium severity (++) to (++++) were obtained with 800  $\mu$ g, + reactions with 400  $\mu$ g and  $\pm$  reactions with 200  $\mu$ g AbN. For the minimal observable reaction ( $\pm$ ) the amount of antibody N required per gram of rat corresponded therefore to approximately 2  $\mu$ g, while for the guinea pig(2) only 0.4  $\mu$ g are required per unit weight.

In the passive cutaneous anaphylaxis experiments a value of 0.5-1.0  $\mu$ g AbN was found for the threshold of the reaction with both the SIII-anti SIII and Ea-anti Ea systems, in agreement with earlier findings(6). The ratios between the amounts of antibody required for minimal reverse Arthus reactions and for passive cutaneous anaphylaxis in the rat and in the guinea pig were respectively 10 and 1000-2000. Since the skin-sensitivity to histamine is about the same in the two species, it is suggested that the higher amounts of antibody required for passive cutaneous anaphylaxis in the rat be ascribed to a lesser ability of the rabbit antibody to become fixed to the dermal tissue.

The enormous difference in the minimal

amounts of antibody required for passive cutaneous anaphylactic reactions, as contrasted with the essentially identical values found for the passive reverse Arthus reaction in the rat and in the guinea pig indicates once again that the two reactions are basically different in their mechanisms.

**Summary.** 1. The severity of the Arthus reaction in the rat was studied using known amounts of antigen and antibody. 2. Reverse Arthus reactions of minimal severity were obtained by intradermal sensitization with 5-10  $\mu$ g anti-Ea or anti-SIII. 3. By intravenous sensitization direct Arthus reactions of medium severity were obtained with 800  $\mu$ g and minimal reactions with 200  $\mu$ g anti-SIII. 4. The threshold of the reaction in passive cutaneous anaphylactic experiments corresponded to 0.5-1.0  $\mu$ g AbN for both the Ea-anti-Ea and SIII-anti-SIII systems. 5. The meaning of these findings is briefly discussed.

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Received November 3, 1952. P.S.E.B.M., 1952, v81.

### Nephrotoxic Globulin Nephritis. III. Prompt Death after Administration of Nephrotoxic Globulin to the Rat.\* (1950)

RICHARD W. LIPPMAN AND E. ELMO JACOBS.

*From the Institute for Medical Research, Cedars of Lebanon Hospital, Los Angeles.*

Some recent studies from this laboratory have concerned the pathogenesis of nephritis produced in the rat by administration of rabbit anti-rat-kidney gamma globulin (nephrotoxic globulin, NTG)(1,2). It was

noted from the start of these studies that NTG administration produced extrarenal effects, such as intravascular hemolysis. The latter could not be eliminated completely by absorption of the NTG with homologous or heterologous erythrocytes. A variable proportion of the animals in each experiment died within the first 12 to 24 hours after NTG administration and, at autopsy, their kidneys

\* This work was supported by a grant from the Research Trust Fund of Los Angeles. Present address of authors: The Bronx Hospital, New York 56, N. Y.