Immunophysiology. Increase in Radiosodium Space of Adrenal Glands in Rabbits Sensitized with Human Plasma.* (19335)

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Since von Pirquet and Schick published their description of serum sickness(1), many studies concerning the relationship of certain histologic findings to immunologic phenomena have been reported(2-5). Such anatomic changes have been well delineated, but the physiologic alterations which are induced by antigen-antibody reactions are still poorly understood. It has been found previously that in rabbits sensitized with human plasma shifts in the distribution of body fluids occur at the time humoral antibody is first detected. The data were interpreted as indicating that a severe immune reaction may be associated with changes in the permeability of membranes (6). The methods employed in these studies did not permit localization of the observed changes to any specific tissues or organs.

The present experiment was undertaken in order to determine the site of the maximum alteration in fluid distribution following the injection of a large dose of human plasma into rabbits. Radioactive sodium (Na^{24}) was used as a tracer.

Materials and methods. Domestic rabbits of mixed breeds, weighing from 2 to 4 kg each, were placed in individual cages and fed a stock diet. Water was given without restriction. The same pool of human plasma was used as the sensitizing antigen and as antigen for the precipitin test. Another pool of human plasma was denatured by being heated to 100°C for 10 minutes. The filtrate of heat-denatured plasma was diluted with physiologic saline solution to the original volume. The methods for the determination of the plasma volume, thiocyanate space, radiosodium space, tissue radiosodium content, and tissue water content have been described previously(7). The appearance of humoral antibody was detected by a qualitative ring precipitin test. The human plasma, diluted 1:100 in physiologic saline solution, was used as antigen to overlay an equal volume of undiluted rabbit serum in 2 x 20 mm test tubes.

Plan of the experiment. Eighteen rabbits were divided into 3 groups of 6 each. *Group 1* (test) and *Group 2* (control) consisted of 6 rabbits each. The animals in the 2 groups were paired as to sex and body weight. On each rabbit, two baseline determinations of the plasma volume, the hematocrit, and the thiocyanate and radiosodium spaces were made 6 to 7 days apart. Each of the animals in Group 1 then received intravenously 10 ml of human plasma per kg of body weight, 5 ml/kg being administered on each of 2 successive days. The animals in Group 2 did not receive injections of human plasma, but were otherwise treated identically. Beginning on the fourth day after the injection of the initial dose of antigen, precipitin tests were performed daily on all rabbits in both groups. On the day when humoral antibody first appeared in an animal in Group 1, the volume determinations were repeated on the animal in Group 2 which had been paired with it, and the 2 rabbits were then sacrificed by exsanguination. For each animal the tissue radiosodium space was determined on 2 portions (roughly 1-2 g each) of the following: cartilage, kidney, skin, tendon, lung, subcutaneous connective tissue, heart, appendix, stomach, adrenal, liver, spleen, abdominal muscle, diaphragm, and gastrocnemius muscle. The total water content was determined on one portion of each tissue. *Group 3,* which served as an additional control, consisted of 6 rabbits whose sex and weight distribution was comparable to that of the rabbits in the preceding two groups. Each animal received by intra-

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venous injection 10 ml of denatured human plasma per kg of body weight, 5 ml/kg being administered on each of 2 successive days. No volume determinations were made in this group, since no changes had been detected in Groups 1 and 2. Six and a half days after completion of the injection of denatured plasma—the average interval at which precipitins were first detected in Group 1—the animals were sacrificed by exsanguination for tissue analyses. At that time the precipitin tests were negative to both denatured and undenatured plasma. The following organs were studied: adrenal, kidney, stomach, and skeletal muscle.

Statistical analysis. The total blood volume, plasma volume, thiocyanate and radiosodium spaces were recorded in milliliters per kg of body weight. For each animal the difference between the mean of the two baseline determinations and the single value obtained on the day the precipitin test first became positive was calculated. The mean difference between the baseline and response values was then obtained, and the significance of the mean difference was tested by the following formulae in which $\bar{d}$ = mean difference, $s\bar{d}$ = standard error of the mean difference, S = summation of, n = number of animals:

$$t = \frac{\bar{d}}{S\bar{d}} \qquad S\bar{d} = \sqrt{\frac{Sd^2 - \frac{(Sd)^2}{n}}{n\,(n-1)}}$$

The calculated value for "t" was compared with the value at (n—1) degrees of freedom, as obtained from standard tables. "t" was considered significant if $P < 0.01$. The results of the tissue analyses were analyzed by groups. The mean values for tissue water content and radiosodium content were obtained for each group and their respective standard errors were then calculated. The significance of the difference between the means was tested by the following formulae:

$$\bar{d} = \bar{x}_1 - \bar{x}_2 \qquad S\bar{d} = \sqrt{S\bar{x}_1^{-2} + S\bar{x}_2^{-2}}$$

$\bar{x}$ = group mean
$S\bar{x}$ = stand. error of mean

Results. Precipitins to undenatured human plasma developed in the animals in Group 1

TABLE 1. Fluid Spaces in Rabbits Given Injections of Human Plasma and in Paired Uninjected Control Animals.

Group			Wt (kg)	Radiosodium space (ml/kg)	Thiocyanate space (ml/kg)	Plasma vol. (ml/kg)	Hematocrit (vol. %)	Total blood vol. (ml/kg)
1	Test animals inj. with human plasma, 10 ml/kg	Baseline	3.07 ± .60	256 ± 15.7	216.5 ± 19.6	41 ± 4.2	40.8 ± 2.4	69.8 ± 8.5
		Response*	3.39 ± .45	261 ± 14.2	221.3 ± 28.8	43.7 ± 8.9	37.8 ± 2.8	70.5 ± 16
2	Controls not inj. with plasma	Baseline	3.57 ± .12	249 ± 11	209.2 ± 10.7	39.8 ± 6.7	40.3 ± 4.1	66.5 ± 9.5
		Response†	3.66 ± .47	249 ± 10.9	207.2 ± 18.9	37 ± 4.05	39.5 ± 3.3	61.5 ± 7.8

All values are expressed as mean ± stand. dev.
* Values obtained on day when humoral antibody first appeared (mean of 6.5 days after initial inj. of human plasma).
† Values obtained at a time interval corresponding with appearance of humoral antibody in Group 1.

(test) at a mean of 6.5 days after the initial injection of antigen. No precipitins to undenatured or denatured plasma developed in the animals in Groups 2 or 3. When the average of the mean baseline values at the time of sacrifice were compared with the average of the mean baseline values, no significant difference was found in the weight, plasma volume, hematocrit, total blood volume, thiocyanate space, or total radiosodium space in any group (Table I).

Comparison of the results of the tissue analyses on the animals in Groups 1 and 2 shows the radiosodium space in the adrenal glands of the sensitized animals to be significantly increased, without an accompanying increase in total water content (Table II). No significant difference in the radiosodium space or the water content of any of the other tissues or organs was found when Group 1 is compared with Group 2.

The results of the tissue analyses in Groups 1 and 3 likewise reveal the radiosodium space to be significantly increased only in the adrenal glands of the animals injected with undenatured plasma as compared with those given denatured plasma. No significant difference in the total water content of the gland was found. The kidney, stomach, and skeletal muscle revealed no change in radiosodium space or total water content.

Comment. The development of humoral antibody in all animals injected with undenatured plasma (Group 1) indicates that a certain degree of sensitization was induced. Since no generalized physiologic alterations developed it must be assumed that this sensitization was minimal. The fact that changes in the tissue radiosodium space were limited to the adrenal glands of those animals injected with untreated human plasma and were not present in those given denatured plasma or no antigen at all suggests that this gland may play some vital role in the maintenance of homeostasis. It may be suggested that the change in the adrenal gland may be a compensatory response to the harmful effects of an antigen-antibody reaction. From the data available, it is not possible to determine whether this increase in the radiosodium space of the adrenal gland was due to interstitial edema, to an increase in the isotope content within the parenchymal cells, to a relative decrease in parenchymal tissue, or to a combination of these factors.

TABLE II. Radiosodium Space and Total Water Content of the Adrenal Gland in Sensitized and Control Rabbits. Six animals in each series.

Group		Mean values: Radiosodium space (% of wet wt)	Water content (% of wet wt)
1	Inj. with undenatured plasma	19.06	63.03
2	Uninj. controls	14.98	65.75
3	Inj. with denatured plasma	11.26	56.80

Summary of statistical analyses

Groups	Radiosodium space $\bar{d}$	$S\bar{d}$	t	Water content $\bar{d}$	$S\bar{d}$	t
1 vs. 2	4.08	.63	6.48*	2.72	4.74	.57
1 vs. 3	7.80	1.32	5.91*	3.72	1.20	3.10
2 vs. 3	3.72	1.20	3.10	8.95	3.94	2.27

* Statistically significant. $P = <.01$.

Summary. Rabbits sensitized with human plasma (10 ml/kg) developed humoral antibody following a mean interval of 6.5 days. No changes were noted in the blood volume or in the thiocyanate or radiosodium space at that time. Tissue analyses revealed the radiosodium space to be significantly increased in the adrenal gland of those animals given injections of untreated plasma, as compared with animals given denatured plasma or no antigen at all. No such increase was noted in any of the 14 other tissues or organs studied. No significant differences in the water content of any of the tissues or organs were found.

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