Release of Slow-Reacting Substance of Anaphylaxis in the Rat: Polymorphonuclear Leukocyte

Abstract. *The antigen-induced release of slow-reacting substance of anaphylaxis was studied in rats previously treated with different biological and pharmacological agents to deplete these animals of specific cellular elements. The polymorphonuclear leukocyte appears necessary as a crucial cell type for optimum release of the slow-reacting substance of anaphylaxis, whereas the mast cell and lymphocyte are not required.*

Slow-reacting substance of anaphylaxis (SRS-A) has been implicated as the chemical mediator of asthma in humans (*1*). It is released from the lungs of sensitized guinea pigs and allergic humans challenged in vitro with specific antigen (*2*). Chemical definition of this mediator has not been achieved, but some evidence suggests that it is an acid-lipid (*1*). In biological assays, SRS-A behaves quite characteristically and is readily distinguished from histamine, serotonin, and kinins. It contracts the guinea pig ileum and the human bronchial smooth muscle in the presence of an antihistamine without producing tachyphylaxis; it has no effect on the uterus of the estrous rat and is not destroyed by incubation with chymotrypsin.

Some workers have suggested that this substance originates in the mast cell (*3*), but others have questioned this (*4, 5*). Our purpose was to determine the prerequisite cellular elements for formation in vivo and release of SRS-A in the rat (SRS-A^{rat}).

Rapp (*6*) described a method for the immunologic release of rat SRS-A in the peritoneal cavity of the rat. The substance present in the peritoneal fluid was pharmacologically indistinguishable from the SRS-A recovered from perfused, shocked guinea pig lungs (SRS-A^{gp}). We used this technique of intraperitoneal injection to study the release of SRS-A in rats previously treated with various agents to produce depletion of specific cellular elements.

Male Sprague-Dawley rats (200 to 300 g) were passively sensitized by the intraperitoneal injection of 0.6 ml of rabbit hyperimmune antiserum to bovine serum albumin (BSA) containing 5 to 12 mg of specific antibody protein per milliliter. Four hours later, the animals were challenged by the intraperitoneal injection of 2 mg of BSA in 5.0 ml of Tyrode's solution containing heparin (50 μg/ml). The rats were stunned 5 minutes later and exsanguinated from the carotid artery. A 1.0-ml sample of each bleeding was collected in 9.0 mg of disodium ethylenediaminetetraacetate, and another 4 to 5 ml was collected in clean glass tubes. After exsanguination, the abdominal wall was incised and reflected; the peritoneal fluid was aspirated with siliconized Pasteur pipettes and collected in iced centrifuge tubes. The fluid was centrifuged at 150*g* for 4 minutes at 4°C, and the supernatants were collected in iced polypropylene tubes. The sedimented cells were again suspended in 3.0 ml of Tyrode's solution and boiled for 8 minutes to extract the residual cellular histamine and serotonin. Supernatant and cellular histamine were assayed on the standard, isolated preparation of guinea pig ileum in the presence of $10^{-7}M$ atropine (*5*). We assayed rat SRS-A with a similar preparation in which the histamine response had been abolished with $10^{-6}M$ mepyramine maleate. One unit of rat SRS-A was arbitrarily defined as the concentration required to produce a contraction whose amplitude was equivalent to 5 mμg of histamine base in that assay. Representative samples were also assayed for serotonin and bradykinin with the uterus of the estrous rat (*5*); $10^{-6}M$ methysergide (*7*) was used to block the effect of serotonin. Insignificant concentrations of these mediators were released. Blood counts and determinations of whole complement in serum (*8*) were carried out for each rat.

Table 1 gives the various procedures for depleting the rat of specific cell types. The first procedure involved the osmotic disruption of the mast cells of the rat peritoneal cavity by the instillation of 20 ml of distilled water. Fawcett (*9*) described the depletion of mesenteric mast cells of rats with this method; we found it equally effective in obliterating the free peritoneal mast cells as assessed by either the residual cellular histamine concentration or morphologic examination of the peritoneal cells. Groups of rats were also previously treated with a rabbit antiserum directed against rat mast cells (*10*). These rats had virtually no intact free peritoneal or mesenteric mast cells, as determined by the residual cellular histamine concentration or by microscopic examination of sedimented cells and mesentery stained with toluidine blue. In rats devoid of peritoneal mast cells due to prior treatment with distilled water or rabbit antiserum to rat mast cells, generation of rat SRS-A did not differ significantly from that in control animals. Thus, the participation of other types of cells was assessed.

To evaluate the role of the polymorphonuclear leukocyte, leukopenia in rats was produced with nitrogen mustard; this left the peritoneal mast cells intact. Attempts to generate SRS-A in rats previously treated with this drug revealed almost complete suppression of release of rat SRS-A (Table 1). In an attempt to confirm that polymorphs were required for this release, rats were treated with a rabbit antiserum to rat polymorphonuclear leukocytes (*11*). Rats treated with this antiserum became markedly leukopenic

Table 1. Effect of various procedures on serum complement ($C'H_{50}$), total circulating polymorphonuclear leukocytes (PMN), peritoneal mast cells, and antigen-induced release of slow-reacting substance (SRS-A^{rat}). Results expressed as mean of six rats. C, control group; E, experimental group. Modifying procedure: I, distilled water (20 ml, 5 days previously); II, rabbit antiserum to rat mast cells (2 ml, 1 day previously); III, nitrogen mustard (2 mg/kg, 5 days previously); IV, rabbit antiserum to rat PMN (3 ml, 1 day previously). Agents were given intraperitoneally, except for nitrogen mustard which was administered intravenously.

Modifying procedure	Circulation				Peritoneal cavity				
	$C'H_{50}$ (unit/ml)*		Total PMN/ml		Total histamine in free peritoneal cells (μg per rat)		Antigen-induced release of SRS-A (units per rat)		Suppression of rat SRS-A release (%)
	E	C	E	C	E	C	E	C	E	
I	207	289	3325	2520	18.6	0.15	289	362	0
II	234	203	3496	2808	26.0	0.02	677	592	13
III	207	274	3364	177	12.0	10.7	650	15	98
IV	234	161	3825	0	18.0	0.03	558	60	89

* Data on whole complement was obtained in a separate experiment with six rats per group; all other determinations were obtained as part of same experiment.

and released only 11 percent of the rat SRS-A of a control group (Table 1). Experiments with a rabbit antiserum to rat thymic lymphocytes (*12*), given intravenously in a dose of 0.2 ml 4 hours before the experiment, revealed no significant suppression of release of rat SRS-A when the absolute lymphocyte count was reduced to 20 percent of control values.

The number of hemolytic units of complement ($C'H_{50}$) of rats which had produced rat SRS-A was reduced by about 25 percent as compared to a group of control animals. The effect of the various treatments on the $C'H_{50}$ was therefore determined (Table 1). None of these procedures seriously depleted serum complement. When complement was removed from rats by means of a semipurified, nontoxic fraction of venom from cobra (*Naja haje*) (*13*), SRS-A production was markedly reduced. We cannot state whether this inhibition occurred because of dependence of the reaction on complement or because of depletion of the substrate for release of rat SRS-A; cobra venom itself releases a slow-reacting substance from tissue and serum (*14*). Investigation of the possible requirement of complement must await the establishment of a system in vitro.

Stechschulte *et al.* (*5*) successfully produced rat SRS-A in the peritoneal cavity of the rat by use of hyperimmune rat antiserums and specific antigen. In experiments with homologous antiserums in previously treated rats, we confirmed the results obtained with rabbit antiserums. Thus, in the rat, we recognize two different homologous immunoglobulins which mediate the selective release of distinctly different chemical mediators, histamine and rat SRS-A, through the participation of different cell types. One antibody, present early in immunization, sensitizes rat mast cells for the release of histamine and serotonin (*5, 15*), while the other, found in hyperimmune antiserum, prepares the rat for the release of rat SRS-A, provided that circulating leukocytes are present.

The polymorphonuclear leukocyte contains several hydrolytic enzymes which probably participate in the inflammatory process. Acid proteases and cathepsins have been suggested as possible injurious agents (*16*). A cationic protein fraction of lysosomes of polymorphonuclear leukocytes disrupts rat mast cells in vitro, releasing histamine (*17*). Kinin-forming enzyme activity also has been associated with the lysosomal fraction of polymorphonuclear leukocytes (*18*). We stress the significance of this cell in inflammation by regarding it as a prerequisite for the release of a third mediator, rat SRS-A.

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References and Notes

1. W. E. Brocklehurst, *Progr. Allergy* **6**, 539 (1962).
2. ———, *J. Physiol. London* **151**, 416 (1960).
3. B. Uvnäs and I. Thon, *Exp. Cell Res.* **18**, 512 (1959); L. O. Boreus and N. Chakravarty, *Acta Physiol. Scand.* **48**, 1 (1959).
4. K. F. Austen, in *The Inflammatory Process*, B. W. Zweifach, L. Grant, and R. T. McCluskey, Eds. (Academic Press, New York, 1965), p. 587.
5. D. J. Stechschulte, K. F. Austen, K. J. Bloch, *J. Exp. Med.* **125**, 127 (1967).
6. H. J. Rapp, *J. Physiol. London* **158**, 35P (1961).
7. UML 491 was obtained from Merck Sharp and Dohme.
8. J. F. Kent, S. C. Bukantz, C. R. Rein, *J. Immunol.* **53**, 37 (1946).
9. D. W. Fawcett, *Anat. Rec.* **121**, 29 (1955).
10. M. D. Valentine, K. J. Bloch, K. F. Austen, *J. Immunol.*, in press.
11. Dr. C. G. Cochrane supplied us with the rabbit antiserums to rat PMN leukocytes.
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13. Dr. R. A. Nelson contributed the cobra venom factor.
14. Y. Gabr and N. Amin, *Brit. J. Pharmacol.* **29**, 80 (1967).
15. K. F. Austen, K. J. Bloch, A. R. Baker, B. G. Arnason, *Proc. Soc. Exp. Biol. Med.* **120**, 542 (1965).
16. H. Z. Movat and T. Uriuhara, *Exp. Mol. Pathol.* **5**, 539 (1966).
17. A. Janoff, S. Schaeffer, J. Sherer, M. A. Bean, *J. Exp. Med.* **122**, 841 (1965).
18. L. M. Greenbaum and K. S. Kim, *Brit. J. Pharmacol.* **29**, 238 (1967).
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Genetic Recombination in Escherichia coli: Clone Heterogeneity and the Kinetics of Segregation

Abstract. *Several recombinant types may be produced from a single* Escherichia coli *zygote when the donor parent is Hfr-Hayes and segregation is delayed; usually only one type is produced with Hfr-Cavalli. Statistical experiments confirm well-known pedigree analyses by micromanipulation and suggest that DNA synthesis is required before final genetic integration.*

Lederberg (*1*) and Anderson (*2*) using micromanipulation have made pedigree analyses of the progeny of zygotes of *Escherichia coli*; these are among the most revealing studies on genetic recombination. Lederberg found that segregation following matings with an Hfr-Cavalli (HfrC) donor strain was usually complete by the third division and that generally only one recombinant class was produced per zygote. However, with an Hfr-Hayes (HfrH) donor strain, Anderson found that segregation was much more delayed (three to more than nine divisions) and usually several recombinant classes were produced from a single zygote. Tomizawa (*3*) has used a statistical technique to study the kinetics of segregation and has confirmed the findings of Lederberg for matings with an HfrC donor parent. These studies are of particular importance in that they provide limitations on possible mechanisms for genetic integration.

Because of the differences mentioned above, the number of recombinant classes appearing in single clones (clone heterogeneity) and the kinetics of segregation for both HfrC and HfrH donor strains have been studied with a statistical method of analysis. The results confirm the observations of both Lederberg and Anderson and indicate that segregational patterns are dependent on the male strain utilized.

Donor strains were prototrophic for the markers used and were streptomycin-sensitive. For HfrH the order of marker transfer was origin, threonine, leucine, galactose, tryptophan, histidine, and streptomycin; for HfrC, the order was origin, lactose, proline, leucine, threonine, arginine, and streptomycin. The recipient strains (PA309 and PA330) could not synthesize the amino acids or ferment the sugars listed. My results are independent of the recipient strains used.

The general techniques for mating and for determination of the genetic constitution have been described (*4*). Both parental types were grown to concentrations of about 2×10^8 cells per milliliter (exponential phase), mixed in the ratio of 1 male cell per 10 female ones,