DG. The findings are interpreted to indicate increased activity in both types of cells and the underlying regulatory mechanisms are discussed.

1. Landau, B. R., Lubs, H. A., PROC. SOC. EXP. BIOL. AND MED., 1958, v99, 124.

2. Hökfelt, B., Bygdeman, S., *ibid.*, in press.

3. Hultman, E., *Nature*, 1959, v183, 108.

4. Hultquist, G., *Acta Pathol. et Microbiol. Scand.*, 1959, v47, 153.

5. Volk, B. W., Lazarus, S. S., *Diabetes*, 1960, v9, 264.

6. Logothetopoulos, J., Sharma, B. B., Salter, J. M., Best, C. H., *ibid.*, 1960, v9, 278.

7. Unger, R. H., Eisentraut, A. M., McCall, M. S., Madison, L. L., *J. Clin. Invest.*, 1960, v39, 1036.

8. Hökfelt, B., Hultquist, G., *Acta Physiol. Scand.*, 1958, v43, 8.

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Differential Anaphylactic Susceptibility of Chicken and Guinea Pig Intestine.* (26536)

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The possibility of using the intestine of chickens, instead of that of guinea pigs, in the Schultz-Dale reaction was investigated. Since we were unable passively to sensitize the chicken intestine, a study was made of factors that might contribute to the differential anaphylactic susceptibilities of chickens and guinea pigs.

Materials and methods. White Leghorn chickens, weighing 1.3 to 1.7 kg, were given a single intravenous sensitizing injection of chicken or rabbit antiserum containing 25 mg of antibovine serum albumin (anti-BSA) antibody per kg body weight. Both antiserums contained 1.5 mg of precipitating antibody protein per ml of serum as estimated by the quantitative precipitin test (8% salt concentration used for chicken serums)(1). Chickens were fasted for 16 to 18 hours, killed by a blow on the head, and 15 cm of the lower portion of the small intestine cranial and adjacent to the caecum was carefully removed and kept moistened at all times with modified Tyrode solution(2). The intestinal contents were carefully removed by flushing the intestine with 20 ml of fresh Tyrode solution. For each experiment, one control and one experimental strip, 4 cm in length, were immersed in a 120 ml muscle bath of the Schultz-Dale apparatus(3) containing Tyrode solution at 40°C and a stream of O^2 (90%) plus CO_2 (10%) was used to aerate and stir the bath. The intestinal strips were allowed to equilibrate in the bath for 15 minutes, then their reactions were tested by adding 9 mg of BSA, 25 to 800 μg (in 2-fold increments) of histamine, and 0.1 to 0.8 μg (in 2-fold increments) of acetylcholine chloride. After each test the bath was drained, the intestinal strips washed, and fresh Tyrode solution added and equilibrated for 8 minutes.

Essentially the same procedure was followed for the guinea pig. Guinea pigs weighing 0.4 to 1 kg were divided into 5 groups. The first 2 groups received 0.25 and 0.5 mg of rabbit anti-BSA serum per kg body weight, respectively, and the third group received 25 mg of chicken anti-BSA serum per kg body weight. The other 2 groups, which served as controls, were injected with normal rabbit and chicken serums, respectively. The contractility of the ileum, 4 cm in length, was tested with 9 mg of BSA, 0.025 to 0.2 μg (in 2-fold increments) of histamine, and 0.01 to 0.08 μg (in 2-fold increments of acetylcholine in the Schultz-Dale bath maintained at 38°C.

Results. As indicated in Table I, 25 mg of either rabbit or chicken antibody per kg

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TABLE I. Schultz-Dale Reactions of Passively Sensitized Guinea Pig and Chicken Intestine.

	Sensitizing antiserum	Antibody inj., mg/kg body wt	Response to BSA, No. animals positive/No. tested	Minimum dose required for contraction in 120 ml bath: Histamine, μg	Minimum dose required for contraction in 120 ml bath: Acetylcholine, μg
Guinea pig intestine	Rabbit anti-BSA	.25	0/2	.1, .1	.04, .04
		.5	3/3	.1, .1, .2	.04, .04, .08
	Control*		0/2	.1, .1	.04, .04
	Chicken anti-BSA	25	0/3	.05, .1, .1	.02, .04, .04
			0/2	.05, .1	.04, .08
			Avg	.1	.04
Chicken intestine	Rabbit anti-BSA	25	0/3	100, 400, 400	.4, .4, .4
	Control*		0/2	50, 100	.1, .4
	Chicken anti-BSA	25	0/3	50, 200, 800	.2, .8, .4
	Control		0/2	100, 100	.4, .8
			Avg	200	.4

* Controls received equivalent amounts of normal serum.

body weight failed to sensitize the chicken intestine. Attempts to sensitize the guinea pig intestine with 25 mg of chicken antibody per kg body weight also failed. On the other hand the guinea pig intestine was successfully sensitized with as little as 0.5 mg of rabbit antibody per kg body weight.

Guinea pig intestine contracted with an average of 0.1 μg of histamine, whereas chicken intestine required an average of 200 μg. The intestinal strip of the guinea pig also showed higher sensitivity to acetylcholine than did that of the chicken: an average of 0.04 in guinea pigs *vs.* an average of 0.4 μg in chickens.

Discussion. Early in this century, it was reported that the chicken antiserum lacked the "necrotizing effect" on guinea pig tissues which the rabbit antiserum possessed(4), and that rabbit anti-sheep serum did not passively sensitize chickens(5). Recently Ovary(6) found that chicken anti-rabbit gamma globulin serum would not sensitize the guinea pig for passive cutaneous anaphylaxis (PCA) test but that the reverse PCA test would succeed using chicken antibody and rabbit gamma globulin as an antigen, which, according to the author, can be "fixed" by guinea pig tissues.

It appears from the above that the failure of the chicken antibody to sensitize the guinea pig intestine in our experiment may be due to a lack of affinity between chicken antibody and guinea pig intestine. The inability of rabbit or chicken antibody to sensitize chicken intestine can also be explained similarly. However, there are other factors to be considered; Ishizaka, Ishizaka, and Campbell (7) have shown that toxicity of the antigen antibody complex is dependent upon ratio of the antigen to antibody. Accordingly, it may be that the negative cases reported here may be due to failure of the complexes to assume the proper antigen to antibody ratio. The same authors(8) reported that soluble complexes composed of chicken antibody and rabbit gamma globulin did not confer cutaneous anaphylaxis in the guinea pig. However complexes formed *in vivo* may be of a different nature than those formed *in vitro,* as indicated by the apparent contradictory results obtained by Ovary and by Ishizaka *et al.*

The low histamine and acetylcholine sensitivity of the chicken intestine suggests that even if the toxic complex did form, the chicken intestine may not contract due to insufficient amounts of mediators liberated. Makinodan *et al.*(9) reported that uniform fatal anaphylactic shock did not occur in actively sensitized chickens unless the circulating precipitin titer was greater than 7 mg§

§ The original data given were 0.75 mg antibody N/ml as determined in 1% saline. This was multiplied by a factor of 1.5 since antibody content increases by the factor when determined in 8% saline (1).

anti-BSA protein per ml serum. The total amount of circulating antibody would have been about 420 mg in a one-kg chicken. To study passive anaphylaxis, one would have to inject several times this amount to secure an antibody titer of 7 mg antibody/ml serum one or 2 days after injection. Guinea pigs can be passively sensitized, with fatal anaphylaxis, with 0.75 mg of antibody per kg body weight, and circulating antibodies are no longer detected when challenged 2 days later(10). From the above comparison, the difference in susceptibility between guinea pig and chicken to passive sensitization and the difference in histamine sensitivity of the 2 animals appear to be of similar magnitude. Further study on the passive anaphylaxis of the 2 animals is desirable to clarify this point.

Summary. Tested by the Schultz-Dale Technic, chicken antibody failed to sensitize the chicken or the guinea pig intestine. Rabbit antibody sensitized guinea pig intestine but failed to sensitize chicken intestine. The minimum doses of histamine and acetylcholine required for contraction of the chicken intestine were considerably larger than those required for guinea pig intestine.

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1. Goodman, M., Wolfe, H. R., Norton, S., *J. Immunol.*, 1951, v66, 225.
2. Feigen, G. A., Campbell, D. H., *Am. J. Physiol.*, 1946, v145, 676.
3. Campbell, D. H., McCasland, G. E., *J. Immunol.*, 1944, v49, 315.
4. Uhlenhuth, P., Haendel, Z., *Z. Immunitatsforsch.*, 1909, v3, 284.
5. Friedberger, E., Hartoch, O., *ibid.*, 1909, v3, 581.
6. Ovary, Z., *Immunology*, 1960, v3, 19.
7. Ishizaka, K., Ishizaka, T., Campbell, D. H., *J. Exp. Med.*, 1959, v109, 127.
8. ———, *J. Immunol.*, 1959, v83, 105.
9. Makinodan, T., Wolfe, H. R., Goodman, M., Ruth, R., *ibid.*, 1952, v68, 219.
10. Kabat, E. A., Landow, H., *ibid.*, 1942, v44, 69.

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Effect of 6-Mercaptopurine on Homograft Reaction in Rats. (26537)

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Attempts at modification of the immune response to skin homografts by means of physical agents such as x-irradiation, cortisone and nitrogen mustard have been relatively unsuccessful. Recently, Schwartz and his associates(1-3) suppressed the immune response to soluble foreign protein in rabbits with 3 to 6 mg/kg/day of the antimetabolite 6-mercaptopurine (6-MP). Meeker and his associates(4) reported a significant increase in skin homograft survival in rabbits by using 6 to 12 mg/kg/day of 6-MP administered for relatively short periods. However, Hubay *et al.*(5), using doses up to 24 mg/kg/day in rats for short periods of time, found no prolongation of homograft survival. The present study was designed to evaluate further the effect of 6-MP on the homograft reaction in rats by increasing the dosage to the maximum tolerated level and by long term administration of the drug prior to grafting.

Method. Skin homograft survival was studied in 241 young male rats of the Long-Evans strain which were given freshly prepared 6-MP* intraperitoneally. Twenty additional rats were used as controls. The injection period before homografting varied between 0 and 174 days (Table 1). The drug was administered after grafting until the homograft had been completely destroyed. The rats were divided into 12 groups and were given 6-MP in dosages ranging from 20 to

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