

nicotinic acid. In a similar experiment 0.0475 micromoles of nicotinic acid were destroyed and 0.13 micromoles of oxygen were taken up. This corresponds to 2.7 moles of oxygen per mole of nicotinic acid.

Sodium azide and hydroxylamine inhibited the oxidation drastically, as is seen in Fig. 2. The manometric measurements were verified with the colorimetric method. In another test, the detergent, lauryl pyridinium chloride^{||} in a concentration of 0.0013%, brought about complete inhibition. Octyl alcohol and toluene inhibited the oxidation 68 and 80%, respectively, as indicated with the colorimetric method. There was no oxygen uptake with nicotinamide as substrate.

Summary and Conclusion. The destruction

^{||} We wish to thank the Hooker Electrochemical Corporation, Niagara Falls, N.Y., for a sample of lauryl pyridinium chloride.

of nicotinic acid by *Pseudomonas fluorescens*, previously described by Koser and Baird³ was found to be an enzymic oxidation. Utilization of nicotinic acid is accompanied by oxygen uptake at a ratio of about 3 oxygen to 1 nicotinic acid. Nicotinamide is not attacked by the oxidase under our experimental conditions. Two types of inhibitors interfere with the enzymatic destruction of nicotinic acid: inhibitors of heavy metal enzymes, such as sodium azide and hydroxylamine, and surface active agents, such as octyl alcohol, toluene and lauryl pyridinium chloride. The rate of oxygen consumption indicates a destruction of the nicotinic acid molecule. Since nicotinic acid serves as a substrate and not as a co-enzyme it should be noted that this reaction sets it apart from its known function in the co-factors.

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Effect of Age in Guinea Pig on Local Passive Sensitization and Skin Reactions Toward Histamine.

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Young guinea pigs are universally accepted as being more suitable for the study of anaphylactic reactions than old animals. Thomsen,¹ who confirmed the work of others, found old guinea pigs more difficult to sensitize than young guinea pigs. He also studied the effect of age on the passive sensitivity developed after injection of anti-horse rabbit serum. Sensitivity was measured by injecting the antigen intravenously. His results showed that the young guinea pigs became more sensitive than the old guinea pigs.

To determine whether age had a similar effect on the production of local anaphylaxis, a comparison was made of the ability to transfer sensitivity passively to the skin of old

and of young animals. The method recently described by Chase² for producing local passive sensitization in the skin of the guinea pig was used for making these comparisons.

Since a tissue liberation of histamine or histamine-like substance is thought to occur during anaphylactic reactions,^{3,4} the response to intradermal injection of this substance in young and in old guinea pigs was studied.

Material. All of the animals were albinos and were obtained from a single breeding colony. Animals of both sexes were used. The old animals were 2 to 4 years and the young

² Chase, M. W., *Proc. Soc. Exp. Biol. and Med.*, 1943, **52**, 238.

³ Dale, H. H., and Laidlaw, P. P., *J. Physiol.*, 1910, **41**, 318.

⁴ Lewis, T., *Brit. M. J.*, 1926, **2**, 61.

¹ Thomsen, Olof, *Z. f. Immunitats. forsch.*, 1917, **26**, 213.

TABLE I.
Local Passive Transfer of Anti-protein Serum to the Skin of Young and of Old Guinea Pigs.

Exp. No.	No. of animals	Mean wt	Age	Dose of anti-protein serum	Latent period before injecting antigen, hr	Mean dose of antigen inj. intraabdom.	No. with positive reactions	% positive	Mean dimensions of reactions in mm	System
1	12	553	10 wks	0.1 cc undil. serum	24	126 mg	8	67	21.6x24.1x1.1	Azoovalbumin-anti-azovalbumin
	12	960	2-4 yrs	"	24	189 "	0	0	0	"
2	5	487	8 wks	0.05 cc 1:4 dilution	24	1.0 cc	5	100	17.8x18.4x1.3	Horse serum-anti-horse serum
	5	1159	2-4 yrs	"	24	2.3 "	1	20	8.0x11.0 f*	"
	10	948	2-4 "	"	48	1.6 "	2	20	8.5x10.0 f	"
3	5	477	8 wks	"	96	1.0 "	2	40	11.0x12.0 f	"
	5	1158	2-4 yrs	"	96	2.3 "	1	20	8.0x8.0 f	"
4	5	398	6 wks	0.1 cc 1:2 dilution	24	0.6 "	4	80	23.8x26.0x1.5	"
	5	1066	2-4 yrs	"	24	1.7 "	1	20	18.0x22.0x0.5	"
5	5	317	5 wks	"	24	intradermally 0.1 cc undil. horse serum	5	100	normal site 21.0x22.8x2.0 sensitized site†	"
	10	1193	2-4 yrs	"	24	"	3	30	36.0x40.5x5.0 normal site 16.0x19.3x3.3 sensitized site 20.3x24.3x1.0	"

* Erythema only, no edema.

† Mean dimensions in animals in which reactions were elicited.

or young adult animals were 4 to 10 weeks of age.

Anti-azoovalbumin guinea pig and anti-horse guinea pig serum were the antisera employed. The method described by Heidelberger, Kendall, and SooHoo⁵ was followed for preparing the azoovalbumin. The anti-azoovalbumin was prepared by injecting 50 mg of the antigen intraabdominally into old guinea pigs. The animals were bled 44 days after the sensitizing dose of antigen was given. The anti-horse guinea pig serum was prepared by injecting 0.01 cc of horse serum intraabdominally into 600 g guinea pigs. These animals were bled 21 days after administration of the antigen.

The histamine dihydrochloride solutions were freshly prepared in saline just before use.

Experimental. The antisera were injected into the closely clipped skin of the backs of both the young and the old animals. After varying intervals of time the corresponding antigens were injected. In experiments 1, 2, 3, and 4 (Table I) these antigens were administered intraabdominally. The dose of azoovalbumin administered to the young animals was 126 mg. The old animals received 189 mg. The dose of horse serum injected was 0.15 to 0.2 cc for each 100 g of body weight. The reactions were measured 15, 30, 45, 60, 90, and 120 minutes later. In most instances they were fully developed or reached their maximum intensity in 60 minutes; consequently, the mean diameters of these 60-minute values are the only measurements given. In agreement with Chase, reactions were not elicited in all of the young animals. The data given in Table I show that the skin of young guinea pigs is more easily sensitized than the skin of old guinea pigs.

Experiment 5 (Table I) was conducted differently than the first 4 experiments because we thought it conceivable that the absence or low sensitivity in old animals could be accounted for by a slow rate of

absorption of horse serum. In this experiment, 0.1 cc of a 1:2 dilution of anti-horse guinea pig serum was injected on one side of the back. For a control 0.1 cc of a 1:2 dilution of normal guinea pig serum was injected into a corresponding site on the other side of the back. Twenty-four hours later, 0.1 cc of horse serum was injected intradermally into each site. The two reactions were read 15, 30, 45, and 60 minutes after administration of the antigen. The skin was considered to be sensitized in those animals in which edema occurred at the sites previously injected with the antiserum. In these animals, the skin reactions at the sites of the injections of the antiserum were significantly larger than the blebs formed by the injections of normal horse serum.

The results of these experiments show that there was no significant difference in the number of old animals that reacted whether the antigen was given intradermally at the site of injection or given intraabdominally.

Activity of histamine dihydrochloride injected into the skin of old and of young guinea pigs. Three groups of old and young closely clipped guinea pigs were injected intradermally with 0.1 cc of varying dilutions of histamine (Table II). The histamine was injected on one side and 0.1 cc of saline, for control, was injected at the corresponding site on the other side of the back. The pH of the 1:10,000 dilution of histamine was 4.8. The pH of the saline control for this dilution was brought to this value by the addition of Sorensen's sodium citrate-hydrochloric acid buffer pH 4.8 in the proportion of one part of buffer to 39 parts of the saline. The pH of the 1:100,000 and 1:1,000,000 dilutions of histamine was approximately that of the saline used for the control injection and consequently needed no adjustment. The reactions, localized edematous areas, were read 15, 30, 45, and 60 minutes after injection. All of the old and young animals injected with the 1:10,000 and 1:100,000 dilutions of histamine reacted. It was observed that the reactions, in most instances, reached their maximum intensity 30 minutes after injection. The 30-minute readings, being the most

⁵ Heidelberger, M., Kendall, F. E., and SooHoo, C. M., *J. Exp. Med.*, 1933, **58**, 137.

TABLE II.
Cutaneous Response to Intradermal Injection of Various Concentrations of Histamine Dihydrochloride in Young and Old Guinea Pigs.

No. of animals	Mean wt	Age	Dose	Mean size of reactions in mm		Ratio of areas
				Histamine	Saline	
5	337	5 wk	1:10,000	22.4x24.4	14.0x14.4	2.7
5	1080	2-4 yr	"	16.6x19.6	10.2x13.4	2.4
10	375	6 wk	1:100,000	17.3x19.6	13.4x15.4	1.6
10	1080	2-4 yr	"	16.6x17.7	12.5x14.5	1.6
5	421	7 wk	1:1,000,000	13.8x15.8	13.6x15.4	1.0
5	1145	2-4 yr	"	14.6x15.2	13.8x15.0	1.1

satisfactory, were the only ones given consideration for purposes of comparison. The injections of saline into the skin of young animals and that of old animals does not produce the same size blebs. For this reason, in analyzing the data from the experiment, the size of the bleb formed by injection of the saline control must be considered in relation to the size of the corresponding histamine wheal. Comparison between the ratios of the areas of the saline and histamine wheals observed in old and in young animals is made, therefore, rather than a comparison of the areas themselves. On this basis of comparison there is no significant difference between the reactions elicited to histamine in the old and in the young guinea pigs.

Discussion. The foregoing experiments show that there is a marked difference in the ability of anti-protein serums to sensitize the skin of old and of young guinea pigs.

The reactions in the two age groups toward histamine were studied to determine whether a weaker response toward histamine could account for the difference in reactivity. The results show that both old and young guinea pigs react in the same degree. It is of interest to note that Lamson and Pope⁶ and Darsie,

*et al.*⁷ were unable to produce reactions in the skin of the guinea pig with concentrations of histamine as high as 1:100. Ramsdell⁸ on the other hand, demonstrated reactions with a 1:1,000,000 dilution of histamine.

We have no explanation for the inability of Lamson and Pope and of Darsie, *et al.* to obtain positive reactions to histamine other than that suggested by Follansby and Hooker⁹ that some strains of guinea pigs may be more reactive toward histamine than others.

Summary. The skin of old guinea pigs is more difficult to sensitize passively than the skin of young guinea pigs. This difference in response is not due to differences in the reactivity of old and of young guinea pigs toward histamine since both old and young guinea pigs react toward this substance to the same degree.

⁶ Lamson, R. W., and Pope, M. L., *J. Immunol.*, 1927, **14**, 365.

⁷ Darsie, M. L., Perry, S. M., Rosenfeld, D., and Zaro, J. A., *PROC. SOC. EXP. BIOL. AND MED.*, 1945, **59**, 278.

⁸ Ramsdell, Susan G., *J. Immunol.*, 1928, **15**, 305.

⁹ Follansby, E. M., and Hooker, S. B., *J. Immunol.*, 1944, **49**, 353.