TABLE I.
Average Values Obtained in Testing the Curative Effect of Vitamin H for Egg-White Injury in Rats, by Oral and Parenteral Administration.

Preparation Tested	Source	Rat day dose administered: Orally, ml	Parenterally, ml
K-5	yeast	0.5	0.1
K-646	"	0.3	0.07
H-29	"	0.3	0.05
H-33	"	0.5	0.1
H-34	"	0.4	0.1
K-211	liver	0.6	0.2
K-223	"	0.75	0.15
K-554	"	0.6	0.12
H-169a	"	>0.125	0.035
H-4/35	"	0.6	0.2
H-5/35	"	1.2	0.3

fluenced by oral doses were given ½ to ¼ of the same doses parenterally with beneficial effect.

The results of the quantitative experiments are summarized in Table I.

Conclusion. The factor (vitamin H) curative of egg-white injury in rats is 3 to 5 times more effective in parenteral than in oral administration.

11100

Immediate Reactions, to Anhydrides, of Wheal-and-erythema Type.*

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Immediate skin reaction in guinea pigs sensitized to proteins were observed by Zinsser.[1] Similar reactions in pigs sensitized to protein compounds of acyl chlorides have been produced by Landsteiner and Jacobs[2] although immediate reactions to acyl chlorides themselves were not observed. The present report deals with the production of

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1 Zinsser, H., *J. Exp. Med.*, 1921, **34**, 495.

2 Landsteiner, K., and Jacobs, J., *J. Exp. Med.*, 1936, **64**, 625.

immediate reactions in guinea pigs, with simple chemical compounds of the hitherto neglected anhydride group.

The examination of anhydrides for capacity to sensitize guinea pigs was prompted by Kern's observation of sensitivity to phthalic anhydride in a chemist.[3] With this substance, contrary to the usual experience in similar cases, it was easy to induce definite sensitization in guinea pigs.

Following this lead, a number of anhydrides were examined. For sensitization, three or more albino guinea pigs were injected on the back twice a week, intracutaneously, with 0.05 cc of a 0.1% olive-oil solution of the respective compounds. In the case of phthalic anhydride it was necessary first to dissolve 20 mg in 0.1 cc dioxane, subsequently diluting with 1.9 cc of olive oil. Treatments were then continued for 2 or 2½ weeks, and the animals tested not more than 2 weeks after the last treatment.

Definite sensitization was observed in animals injected with citraconic, phthalic, 3-nitrophthalic, and n-caproic anhydrides. Of these compounds, citraconic anhydride gave by far the most striking results, described below.

Tests for sensitivity to citraconic anhydride were carried out on the animal's flank, from which the hair had been removed with an electric shaver, by placing a drop of a 25% solution of citraconic anhydride in dioxane on the skin and scratching through the drop, as in human scratch-tests, with a straight needle. In adequately sensitized animals a reaction appeared after a few minutes consisting of a faint to pale pink wheal ranging from several millimeters to several centimeters in diameter (Table I) with pseudopodial extensions and neighboring pinkish, elevated islands in the more reactive pigs. Reactivation of old injection-sites was also observed and the erythema was quite definite when animals were highly sensitive, being strongest 20 to 30 minutes after testing. Within an hour or two these reactions usually lost almost all color, although edema persisted until the delayed reactions developed.

Strong pink to red delayed reactions began to appear 6 to 8 hours after the test and were at their best on the following day. The occurrence of immediate reactions seems to be influenced by the interval between the last treatment and testing, and other factors, but delayed reactions were regularly present. The most striking reactions observed were several times the size of those recorded in Table I.

To determine the specificity of the above effects, guinea pigs sensitized with 3.5-dinitrobenzoyl chloride were used which gave imme-

[3] Kern, R. A., *J. Allergy*, 1939, **10**, 164.

TABLE I.

Specificity of Wheal-and-erythema Reactions to Citraconic Anhydride.

For the scratch test with citraconic anhydride a 25% dioxane solution was used; 0.1 mg of the serum compound with 3.5-dinitrobenzoyl chloride was injected intracutaneously in a volume of 0.02 c.c. Figures refer to the diameter of the lesions in millimeters.

No. of guinea pig	Treated with	Tested with: Citraconic anhydride			Tested with: 3.5-dinitrobenzoyl chloride-guinea pig serum compound		
1	Citraconic anhydride	8, pale pink, markedly elevated	7, faintly pink, elevated	8, pale pink, elevated	7, almost colorless, elevated	almost negative	2, faintly pink nodule
2		10, pale pink, markedly elevated	10, faintly pink, elevated	25, pink, swollen	5, almost colorless, sl. elevated	almost negative	2, faintly pink nodule
3		6, pink, elevated	7, pale pink, swollen	20, pink, swollen	5, colorless, almost flat	4, colorless, almost flat	2, faintly pink nodule
4	3.5-dinitro-benzoyl chloride	2, pinkness along scratch	3, pinkness along scratch	3, pinkness along scratch	9, faintly pink, markedly elevated	9, faintly pink, markedly elevated	30, faintly pink, swollen
5		2, pinkness along scratch	3, pinkness along scratch	3, pinkness along scratch	8, faintly pink, markedly elevated	10, faintly pink, markedly elevated	19, pale pink, swollen
6		2, pinkness along scratch	4, pinkness along scratch	7, pale pink, elevated	10, faintly pink, markedly elevated	14, almost colorless, swollen	35, faintly pink, swollen
7	Untreated controls	2, pinkness along scratch	2, pinkness along scratch	4, pink, slightly elevated	2, faintly pink, slightly elevated	2, faintly pink spot	2, pale pink nodule
8		1, pinkness along scratch	1, pinkness along scratch	2, pale pink, slightly elevated	5, colorless, almost flat	3, colorless, sl. elevated	2, faintly pink nodule
Read after		20 min	45 min	overnight	20 min	45 min	overnight

diate reactions to a protein-compound of this substance prepared by a method described for *p*-chlorobenzoyl chloride.[2] Cross-tests between animals sensitized respectively by 3.5-dinitrobenzoyl chloride and citraconic anhydride (Table I) demonstrated that immediate reactions occurred only to the homologous substances, and were, therefore, manifestations of specific hypersensitiveness. Neither animals sensitized to citraconic anhydride nor controls gave a reaction when tested with dioxane alone by the scratch method. The 3 animals shown in the table were selected from a batch of 8, all of which had been definitely sensitized by the treatment.

Of guinea pigs treated with other compounds of this group, those receiving phthalic anhydride became markedly sensitive, with immediate reactions of moderate size which were not easily reproducible. The delayed reactions, however, were consistently strong. Animals treated with 3-nitrophthalic anhydride gave definite delayed reactions and only occasional, slight immediate wheals with little flare. In 3 pigs treated with caproic anhydride only the delayed type of sensitization was observed except in one animal where, after 30 minutes, a faint pinkishness was present over the entire surface which the substance had touched (not solely in the vicinity of the scratch).†

Further study of the mechanism of these reactions is indicated, in regard to the possible presence of antibodies, the occurrence of immediate reactions in sensitization to simple compounds of other groups, and the special properties of anhydrides which lend themselves to sensitization of this kind.

Summary. A type of immediate wheal-and-erythema reaction, similar to that observed in human atopic hypersensitiveness, was induced in guinea pigs that had been sensitized and tested with chemical compounds of the anhydride group.

† Diffuse pinkishness appearing within half an hour was observed at the site of application of benzoyl chloride in animals sensitized to this substance, suggesting that other acyl chlorides may give similar early reactions.