TABLE V.
Antagonistic Effect of Aleuronat on Virulence of Staphylococci Induced by Mucin.

Date of exp.	No. of staphylococci injected × 100	Staphylococcus Infection + Mucin			
		Without aleuronat		With aleuronat	
		No. of mice	Survivors	No. of mice	Survivors
3/3/44	61	10	0	4	2
	610	10	0	5	4
5/10/44	65	5	1		
	650	10	4	5	4

Summary. 1. Virulent staphylococci injected in mucin do not produce a characteristic leucocytic reaction in the peritoneal cavity. 2. Staphylococci injected intraperitoneally in mucin remain viable and spread from the abdominal cavity; without mucin, they are rapidly destroyed. 3. Staphylococcus vaccine suspended in mucin does not produce leucocytosis during the first hours after injection. 4. *C. diphtheriae* and meningococcus injected intra-abdominally are influenced in the same manner by the presence of mucin. 5. The leucocytic reaction of aleuronat is inhibited by simultaneous injection with mucin. A later injection of mucin does not destroy leucocytes already present in the peritoneal fluid. 6. The virulence-enhancing effect of mucin can be neutralized by leucocytosis previously induced by aleuronat. 7. All these observations suggest that mucin decreases the resistance of the host to bacterial invasion and imparts to microörganisms of otherwise low virulence the potentialities of highly invasive microbes.

15056

Size of Histamine Wheal in Relation to Dose and to Animal Species.*

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Since the first description of the effects of histamine on the skin, there have been many studies and applications of this local reaction. This investigation concerns the variations in response to intradermal injections of histamine in different species, and also the relation of wheal diameter to the concentration of histamine used.

Eppinger[1] first described histamine urticaria produced by scarification. Sollman and Pilcher[2] obtained wheals with histamine by intradermal injection in man, but not in rabbits or cats. They used semi-quantitative methods to measure this skin reaction, finding that the size of the wheal produced by local administration appeared to be a function of the concentration of histamine used. A minimum reaction was obtained with a concentration of 1:100,000. Since this work, there have been numerous methods devised for the determination and assay of histamine, such as the uterine strip, patch test and chemical methods. However, for rapid

* This investigation was undertaken with the aid of the staff of the Department of Pharmacology of the University of Southern California School of Medicine, Los Angeles, California.

1 Eppinger, H., *Wien. med. Wchnschr.*, 1914, **113**, 1913.

2 Sollman, T., and Pilcher, J. D., *J. Pharm. and Exp. Therap.*, 1917, **9**, 309.

routine analysis these leave much to be desired. In order that biological fluids might be assayed for histamine-like substances, the quantitative variation in wheal size with graded concentrations of histamine was studied. Finally, by use of this method of determination, histamine in the presence of a body fluid was measured.

Method. In order to determine the difference in the local skin reaction of man and various laboratory animals, histamine diphosphate† was injected intradermally into the shaved skin of a guinea pig, rabbit, cat, dog, goat (kid), and man. Three injections consisting of 1/20 cc of 1.0%, 0.1%, and a distilled water control were given to each. The resultant wheals were observed 20 min later, at which time the maximum response occurred. Wheals produced by distilled water were recorded as 0 and larger wheals were rated from + to ++++.

The relationship between wheal size and histamine concentration was determined in the following manner. A series of solutions of histamine diphosphate, 1.0, 0.1, 0.01, 0.001 and 0.0001%, and a distilled water control were injected intradermally into the shaved flanks of recently weaned kids. Two animals were used, series B and C being performed on the same animal with 2 weeks intervening. The maximum wheal diameters were measured after 20 minutes.

The following experiment was designed to show the effect of various concentrations of histamine in the presence of a biological fluid which itself either contained histamine-like substances (H-substance[3]) or some other irritative material. A series of histamine diphosphate solutions similar to that described earlier was made using, in place of distilled water, physiological saline mixed with an equal quantity of the intestinal fluid of a fasted cat. These solutions were injected intradermally in a kid and the resultant wheals were measured after 20 min.

Results and Conclusions. It was found that the various animals tested could be divided into 2 groups: those which showed a marked whealing in response to intradermal injections of histamine, and those which showed only a minimum of traumatic response but no true histamine wheal. Table I. summarizes these data. These results are consistent with Dale's[4] observations that certain animals such as rodents respond to histamine by vasoconstriction while others such as the dog, monkey, and man, by vasodilation. The cat seems to occupy an intermediate position in that it shows a decrease of blood pressure[5] following intravenous histamine injection but no skin whealing following intradermal administration.

TABLE I.
Whealing Produced by Intradermal Injections of Histamine in Various Animals.

	Histamine diphosphate 1.0%	0.1%	Distilled water control
Guinea pig	0	0	0
Rabbit	0	0	0
Cat	0	0	0
Dog	++++	+++	0
Goat	++++	+++	0
Man	+++	++	0

The diameters of wheals produced by intradermal injection in the kid were found to vary directly, within certain limits, with the concentration of histamine as shown by the solid line in Fig. 1.

Below 0.001% the concentration of histamine no longer determined the wheal diameter which was apparently dependent upon some other factor due to trauma, possibly the release of H-substance. Thus injection of 0.0001% histamine elicits a wheal approximately equal in size to one produced by the water control. This provides an interesting correlation with the work of Harris[6] who found by chemical analysis of normal skin an H-substance concentration of 0.0001%. Of note, also, is the fact that the variation in wheal diameter in the lower concentrations is far greater than in the higher concentrations. This can be explained on

† Obtained from the Pfanstiehl Chemical Company.

[3] Best, C. H., and McHenry, E. W., *Physiol. Rev.*, 1931, **11**, 371.

[4] Dale, H. H., *Lancet*, 1929, **1**, 1179, 1233, 1285.

[5] Dale, H. H., and Laidlaw, P. P., *J. Physiol.*, 1910, **41**, 318.

[6] Harris, K. E., *Heart*, 1927, **14**, 161.

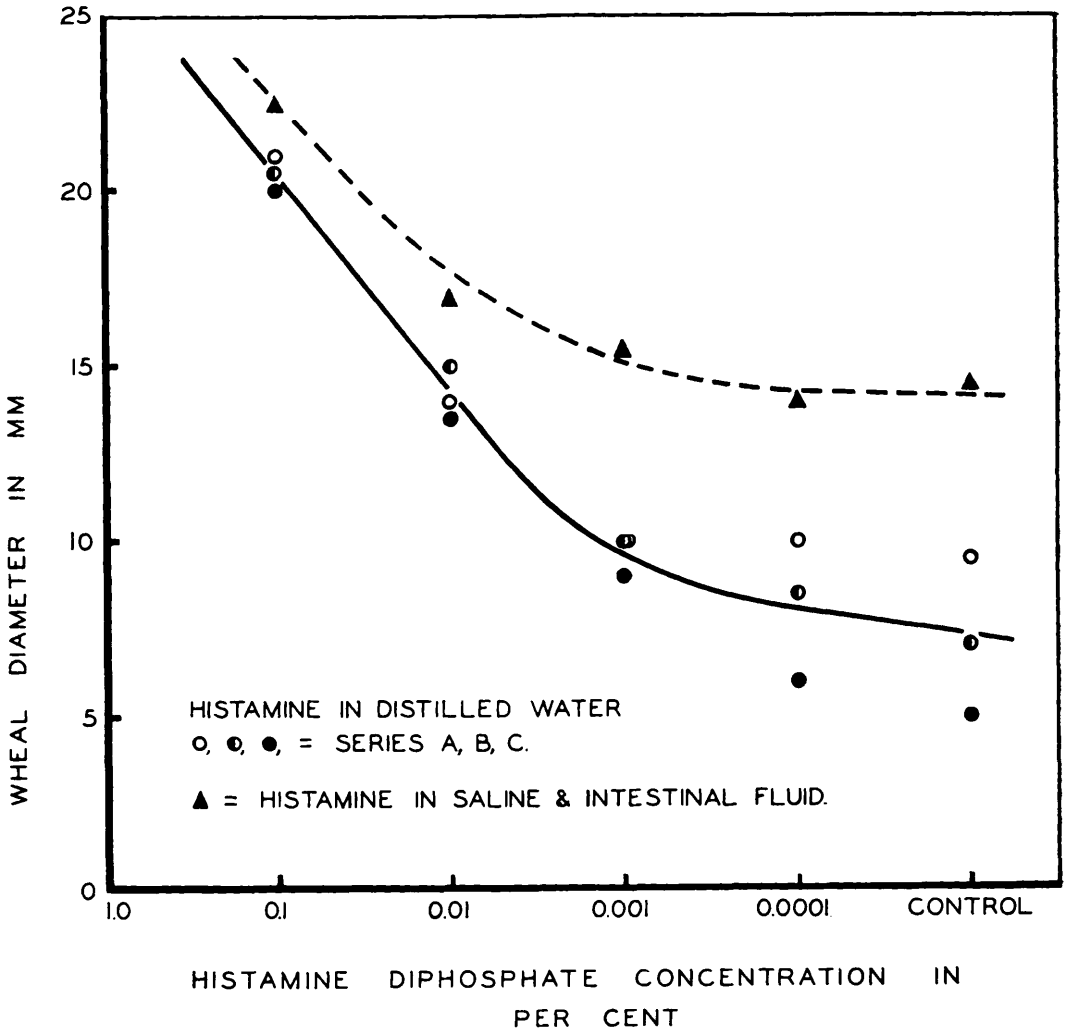

FIG. 1.

The relation of wheal diameter to concentration of histamine diphosphate injected intradermally in the goat. Solid line represents histamine in distilled water; broken line, in a mixture of physiological saline and intestinal fluid from a fasted cat.

the basis of uncontrolled factors causing variation of response to trauma incident to the injection, where histamine is no longer a limiting factor.

Measurement of histamine in the presence of a substance which also produces whealing is feasible providing the concentration of histamine is the limiting factor. Whereas the threshold with histamine alone is 0.001%, in the presence of a histamine-like substance, it is raised to 0.01% as shown by the broken line in Fig. 1. As the histamine diphosphate concentration increases, and the relative amount of H-substance decreases, the size of the wheal becomes increasingly dependent on the former, the 2 curves tending to converge.

These experiments suggest the importance of proper selection of laboratory animals for all dermatological investigations involving a local skin reaction. The response to histamine diphosphate injected intradermally provides a means of selecting animals which have a peripheral vascular mechanism similar to that existing in man. The use of dogs or goats would seem to be more logical than the time worn reliance on guinea pigs and rabbits for the study of response to local irritants and allergenic substances. The quan-

titative relationship existing between histamine concentration and skin wheal size suggests the possibility of using histamine as a standard reference material in comparing various skin irritants.

Summary. There is a species variation in the local reaction to intradermal injections of histamine. The dog, goat, and man show a marked skin whealing, but no such response is obtainable in the guinea pig, rabbit, or cat.

The diameter of the skin wheal produced by intradermal injection is a function of the concentration of histamine used, the threshold being 0.001%. In the presence of an extraneous histamine-like substance, this relationship still exists, but the threshold of histamine sensitivity is elevated to 0.01%.

A practical application to the field of experimental dermatology is suggested involving the proper selection of test animals and the use of histamine as a standardizing agent.

15057

Ulcer Production in Intestines of Dogs by Various Enzymes Under Hydrostatic Pressure.

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In a previous paper from this laboratory the effect of hydrostatic pressure on the production of ulcers was described.[1] In another paper it was reported that ulcers could be produced in the intestines of dogs by an alkaline solution of pancreatin under a hydrostatic pressure of 90 cm H_2O.[2]

The purpose of the experiments reported here was to study the effect produced on the intestines by the following enzymes: rennin, trypsin, erepsin, steapsin, and amylopsin. The enzyme solutions were at pH ranges corresponding to those under which the enzymes act in the normal animal.

The procedure was the same as that described in [1] except that in these experiments only one pressure (90 cm H_2O) was used. The severity of damage to the loops was graded according to a scheme adopted in this laboratory which is described here: (1) represents a condition in which there is a slight surface necrosis of the mucosa; (2) designates a deeper necrosis involving at least $\frac{1}{3}$ of the thickness of the mucosa of the entire area with smaller areas (from $\frac{1}{2}$ cm^2 to 2 cm^2) showing necrosis as deep as the submucosa; (3) indicates necrosis of practically all the mucosa with some patches extending into the circular muscle; (4) was applied to a loop having areas of necrosis extending to the serous coat or to a frank perforation. Each loop was scored in this manner and the average taken for each group of experiments. The results are given in Table I.

Discussion. Rennin (0.1%) in N/10 HCl produced perforating ulcers in 114 minutes while N/10 HCl alone caused perforation in 174 minutes and pepsin (0.1%) in N/10 HCl in 82 minutes.[2] These data show that rennin has a marked digestive effect on the mucosa of the intestine but not so great an effect as pepsin.

Of the other enzymes studied only the proteolytic ones were active. The necrosis produced by trypsin was about twice as severe as that caused by erepsin, but not even trypsin digested the mucosa to an extent comparable to that of rennin and pepsin, and was only about one-half as effective as pancreatin.[2]

Neither steapsin nor amylopsin caused any necrosis of the mucosa, alkaline solutions of these enzymes behaving like controls where the loops of intestine were exposed to the

[1] Driver, R. L., Chappell, R. H., and Carmichael, E. B., *Am. J. Dig. Dis.*, 1945, **12**, 168.

[2] Driver, R. L., *Arch. Path.*, in press.