TABLE II.
Interference of Iodoacetamide with Inhibitory Effect of Epidermal Extract.
Tyrosine, tyrosinase, and buffer same as in Table I. Total volume 13 cc.

Tube No.	Epidermal extract 100 mg/cc cc	Iodoacetamide 24 mg/cc cc	Melanin μg after 24 hrs	48 hrs
1	1.0	0	100	158
2	1.0	1	240	460
3	0.5	0	138	230
4	0.5	1	270	460

ble sulfhydryl compounds in the epidermis. In preliminary experiments the —SH content of our epidermal extracts measured by the ferricyanide method[5] was found to be in the range of 0.0004 millimol of —SH per cc. The —SH content could be substantially depressed by ultraviolet irradiation of excised pieces of skin.

These findings lead to the hypothesis that in melanoblasts both substrate and active enzyme are present but no reaction takes place between them because of the inhibitory action of sulfhydryl compounds. Pigmentogenic stimuli such as sunshine, X-rays, heat and inflammatory cutaneous diseases of unknown origin act by oxidizing or otherwise destroying these compounds, whereupon the enzyme freely acts on the substrate. In addition to postinflammatory pigmentations, hyperpigmentations of endocrine origin may have the same mechanism as suggested by *in vitro* observations of Figge and Allen.[4]

Summary. Aqueous extracts of human epidermis contain substances inhibiting melanin formation in the tyrosine-tyrosinase system. Iodoacetamide interferes with this inhibition. A new hypothesis of the mechanism of postinflammatory pigmentation is presented.

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Significance of Histamine in Trypsin Shock as Determined by Benadryl.*

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The following observations have been interpreted as supporting the hypothesis that trypsin owes its toxicity to the action of histamine which is liberated from tissues by this enzyme. Trypsin, like histamine, causes contraction of isolated segments of ileum and uterus from a number of animals.[1-4] This action, like that of histamine, is not inhibited by atropine but is abolished by arginine.[3-6]

Trypsin, like substances known to liberate histamine, when added to rabbit's blood results in a shift of histamine from cells into plasma.[7,8] Rabbits injected intravenously with trypsin show a leucopenia, a fall in total blood histamine, a fall in carotid and a rise in pulmonary arterial pressures,[3,5,9,10]

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TABLE I.
The Influence of Benadryl on the Toxicity of Trypsin.

Dose trypsin "Difco" (mg/kg)	Control			Treated (10 mg/kg benadryl)		
	No. lived	No. died	% mortality	No. lived	No. died	% mortality
			Guinea Pig.			
80	3	5	63	3	2	40
160	0	4	100	0	4	100
			Dog.			
100	2	7	78	2	7	78

phenomena which are characteristic of the liberation and action of histamine in this species.[11] Trypsin, like histamine and some substances known to release histamine, when injected into the skin of rabbits, gives rise to a positive "trypan-blue reaction."[7,12]

Perfusion of trypsin solutions through the isolated liver of the dog results in the appearance of histamine in the perfusate[13] and the blood of some dogs undergoing trypsin shock shows an elevation in its histamine content.[14]

Perfusion of trypsin solutions through the isolated lung of the guinea pig results in the appearance of histamine in the perfusate.[4,14,15]

There are, therefore, several observations that are consistent with the hypothesis that trypsin owes its toxicity to the liberation and action of histamine. However, there are other observations which are inconsistent with this hypothesis. For example, guinea pigs dying from the intravenous injection of trypsin fail to show either the rise in blood histamine or the emphysematous lungs characteristic of the liberation and action of histamine in this species.[10,16]

The purpose of the present study was to contribute, by the use of benadryl, a potent antagonist to either administered or liberated histamine,[17-20] additional observations bearing on the role played by histamine in the toxicity of trypsin, and to test the adequacy of the existing hypothesis in interpreting these findings.

The trypsin employed in the present studies was a non-crystalline, but potent preparation, sold under the name of "Difco" which has been demonstrated to be free of histamine and to lose all biological activity when treated with trypsin inhibitor from blood plasma. Unless specified it was placed in solution in a borate buffer at pH 7.4.

The first experiments were designed to determine the influence of benadryl on the lethal action of intravenously injected trypsin in the guinea pig and the dog.

Trypsin injected via the jugular vein in a dose of 80 mg per kilo into 8 very lightly anesthetized guinea pigs resulted in the death of 5 or 63% of the animals. Twenty minutes

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TABLE II.
Comparative Susceptibility of Blood Pressure Depressing Actions of Trypsin and Histamine in the Dog to 5.0 mg/kg of Benadryl.

	Trypsin "Difco"			Histamine Acid Phosphate		
		Fall in blood pressure (mm Hg)			Fall in blood pressure (mm Hg)	
Dog No.	Dose, mg/kg	Before Benadryl	After Benadryl	Dose, mg/kg	Before Benadryl	After Benadryl
1	.05	34	46	.002	53	1
2	.05	36	36	.001	40	1
3	.25	58	52	.004	54	22
4	1.00	94	78	.100	80	62
5	.05	64	50	.004	60	22
6	.10	70	66	.004	60	6
7	1.00	39	48	.002	40	18
8	.60	54	58	.004	52	20
Avg		59	54		55	19
% decrease			—8			—65

after the intraperitoneal injection of 10 mg per kilo of benadryl, the same dose of trypsin was injected into another group of 5 guinea pigs with the death of 2 or 40%. In similar fashion 2 groups of 4 guinea pigs were injected intravenously with 160 mg per kilo of trypsin with 100% mortality in both the treated and control groups. (Table I). Autopsy of the animals dying from the injection of trypsin confirms previous observations as to the absence of pulmonary emphysema.

Trypsin injected via the femoral vein in a dose of 100 mg per kilo into 9 pentobarbital anesthetized dogs resulted in a precipitous fall in blood pressure to shock levels with death of 7 or 78%. Following the very slow intravenous injection of 10 mg per kilo of benadryl, the same dose of trypsin was injected into another group of 9 dogs with the death of 7 or 78%.

It may be concluded from these experiments that benadryl affords neither the guinea pig nor the dog any significant protection against the lethal action of intravenously injected trypsin.

These findings are definitely inconsistent with the hypothesis that the action of histamine is the sole factor in the toxicity of trypsin. However, they do not deny the possibility that histamine may be one of multiple non-additive factors in the toxicity of trypsin. On the other hand, the liberation of significant amounts of histamine unaccompanied by any evidence of its action is untenable. Therefore, the next experiments were designed to determine the influence of benadryl on the transient fall in blood pressure produced by sublethal doses of trypsin.

A series of 8 pentobarbital anesthetized dogs, arranged for recording blood pressures, were injected with doses of 0.05 to 1.00 mg/kg of trypsin. These doses caused the blood pressure to fall 36 to 94 mm of mercury with complete recovery within 10 minutes. In each instance the fall in blood pressure produced by trypsin was duplicated by a suitable small dose of histamine phosphate. Five mg/kg of benadryl was then slowly injected intravenously and the original doses of trypsin and histamine were repeated.

A comparison of the influence of benadryl on the blood pressure depression produced by these agents reveals (Table II) that in each instance the reduction in the action of histamine is greater than the reduction in the action of trypsin. In 7 of the 8 animals this difference is striking. The average decrease in the blood pressure response to histamine produced by benadryl is 36 mm or 65% and at the same time the decrease in the response to trypsin is only 5 mm or 8%. It should be stated that a moderate amount of tolerance frequently develops to the repeated injection of trypsin and that such an effect would be included in the apparent decrease in the trypsin response as produced by benadryl.

It may be concluded that benadryl is much less effective in reducing the blood pressure

TABLE III.
Blood Histamine in Trypsin Shock in the Dog.

Dog No.	Treatment	Blood histamine (μg histamine base/cc) Before shock	During shock	Outcome
1	None	.12	.07	Died
2	Benadryl (10 mg/kg)	.05	.15	"
3	None	.13	.08	"
4	Benadryl (10 mg/kg)	.06	.06	Lived

response to trypsin than it is in reducing the response to histamine. These observations are also definitely inconsistent with the hypothesis that the action of histamine is the sole factor in the effects produced by trypsin. They cannot be used to deny any participation of histamine in this effect but can relegate it to a minor role, and thus are in direct contrast to those experiments previously reported which claim a substantial increase in blood histamine during trypsin shock in the dog.

Therefore, an experiment was designed to determine the blood histamine before and during trypsin shock in the dog. Blood was drawn from 4 dogs prior to the intravenous injection of 100 mg/kg of trypsin, a dose large enough to cause death in 3 of them. At the time the blood pressure was falling precipitously as a result of the injection of trypsin another sample of blood was drawn from these animals. The trichloracetic acid filtrate of these bloods was subjected to electrodialysis and concentration and assayed for their histamine content on the blood pressure of the atropinized cat. The histamine content of these bloods was expressed (Table III) in terms of mcg of histamine base per cc. It is concluded that in none of these animals was the blood histamine of sufficient magnitude to explain the severity of the reaction produced.

The present studies, therefore, contribute additional information bearing on the role played by histamine in the toxicity of trypsin and these observations are inconsistent with the hypothesis that trypsin owes its toxicity solely to the liberation and action of histamine. It therefore becomes necessary to reconsider the initial observations which have been interpreted as supporting this hypothesis from the point of view that perhaps other interpretations may be made of some of these facts.

The significance of the observation that arginine, a known histamine antagonist, can abolish the action of trypsin on the isolated guinea pig ileum is dependent upon the specificity of arginine as an antagonist. It has been observed that trypsin can liberate a "slow-reacting muscle stimulant substance" which contributes to the response of smooth muscle to trypsin.[21] The fact that arginine completely prevents the action of trypsin on isolated smooth muscle suggests that the "slow-reacting muscle stimulant substance" may also be antagonized by arginine and does not deny the possibility that trypsin itself may be antagonized by arginine. In any event, the specificity of arginine as a histamine antagonist may be questioned and thus the antagonism of the action of trypsin on smooth muscle by this substance need not necessarily be interpreted as indicating histamine to be the mediator of this response.

The specificity of the positive "trypan-blue reaction" for histamine has never been claimed and, in fact, it is known that substances, such as arginine and Kallikrein, which do not cause the liberation of histamine, give a positive test.[22,23] Therefore, a positive "trypan-blue reaction" produced by trypsin need not be interpreted as indicating the liberation and action of histamine.

The previously reported experiments demonstrating a rise in blood histamine in the dog during trypsin shock are inconclusive

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in that a significant rise was observed in but one dog, and in view of the present observations, these results must be challenged.

The amounts of histamine obtained from the dog's liver by perfusion of this structure with trypsin solutions are very small, being of the order of 5 to 12 mcg in 30 to 45 minutes of perfusion.

The observed liberation of histamine from isolated guinea pig lungs by perfusion of these structures with trypsin solutions appears to be of no consequence to the toxicity of trypsin in the intact animal in view of the absence of any of the pulmonary effects of histamine when trypsin is injected into the intact animal.

There remains only the observations on the action of trypsin on the blood of rabbits and the action on the intact rabbit which clearly support the histamine hypothesis.

Thus, it is concluded that most of the observations which have been interpreted as supporting the hypothesis that trypsin is toxic because of the action of histamine which it liberates from tissues are susceptible of other interpretation. In addition, such an hypothesis is quite inconsistent with the present observations which indicate that histamine plays no significant role in the toxicity of trypsin.

Summary. It was found that benadryl, a potent antagonist to either injected or liberated histamine, does not modify the lethal action of trypsin in the guinea pig or the dog. It is thus apparent that the histamine hypothesis, relative to the toxicity of trypsin, requires reconsideration.

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Studies on the Adherence of the Epidermis to the Corium.

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In some cases of pathologic blister formation, such as epidermolysis bullosa and pemphigus, the separation of the epidermis from the corium cannot be explained by development of pressure due to accumulation of fluid between these layers. In these cases a change in the adhesive forces holding the 2 heterogeneous layers together must be assumed. Histologic studies with modern methods have failed to demonstrate the existence of a basement membrane at the junction of the epidermis and the corium.[1-3] Lately it has been suggested that a decrease in adhesion between the 2 layers is due to physicochemical changes in the upper corium.[4]

It has been known that treatment of the skin with acids or bases results in separation of the epidermis.[5] These types of separation are accompanied by swelling of the corium. In 1942 Baumberger *et al.* discovered that if excised human skin was warmed up to 50°C for a few minutes on a slide warming table, the epidermis could easily be peeled off.[4] When the skin was cooled, the epidermis became reattached to the corium an indication of a reversible gel-sol formation.

Experimental. With regard to the swelling effect of acids, bases, and neutral salts on collagen[6] and the consequent decrease of its

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