tions of penicillin which are almost completely inhibitory, the results further indicate the advisability of using maximal rather than minimal doses of penicillin for the clinical treatment of staphylococcal infections.

Summary. Unstable small colony variants were readily produced from nine out of ten strains of *Staphylococcus aureus* upon exposure to penicillin.

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Observations on the Antagonism Between Heparin and Trypsin.*

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It has been stated that following anaphylactic shock there may be more than the normal amount of antitrypsin in the blood.[1,2,3] The incoagulability of the blood in anaphylaxis has been explained by the discovery that it contains substantial amounts of heparin,[4] and reports that heparin has antitryptic properties[5,6] have led to the suggestion that this substance may be responsible for the increase in the antitryptic activity of such blood.

We have observed that heparin inhibits the histamine releasing effect of trypsin on rabbit blood cells,[7] and that it protects rats from the lethal effects of intravenously injected trypsin.[8] Our observations were interpreted as supporting the view that heparin has a specific antitryptic action. However, the recent demonstration that the blood of heavily heparinized dogs has no increased antiproteolytic activity[9] necessitates a reconsideration of such a view, and has led to the additional studies reported here on the antagonism between heparin and trypsin.

The intravenous injection of heparin (10-15 mg per kilo) did not modify the fall in blood pressure produced by the intravenous injection of trypsin (0.1-1.0 mg per kilo) into anesthetized dogs (4 experiments). Nor did the incubation of heparin with trypsin for various periods of time prior to injection alter its depressor activity. On the other hand, the trypsin preparation used ("Difco") was inactivated when incubated with blood serum, which is known to be antitryptic. These results, therefore, seem valid and suggest that heparin is not a specific antitryptic agent.

Accordingly, it was thought necessary to reinvestigate the *in vitro* antitryptic action of heparin by means of a standard enzyme method. The Anson-Mirsky hemoglobin digestion method was used. Mixtures of heparin and trypsin were incubated at 25°C. At various intervals a 5 cc fraction of the mixture was added to the hemoglobin substrate and the tryptic activity determined. Mixtures of trypsin with serum were incubated and

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We are indebted to Roche-Organon, Inc., for the supply of Heparin (Liquaemin) used in our experiments.

The fastusol was obtained from Central Scientific Co., Chicago, Ill., as Chlorazol Fast Pink B.

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TABLE I.
Influence of Various Agents on Proteolytic Activity of Trypsin.

Inhibiting agent (vs. trypsin 2.25 mg)		pH†	Activity* before inhibitor was added	Activity* remaining after incubation with inhibitor for 5 min	10 min	15 min	20 min	25 min	30 min	60 min
Heparin	1.25 mg	7.5	1.07	1.24	1.24	1.15	1.35	1.30	1.20	
"	12.50 "	7.5	0.92	1.10	1.20	1.10	1.10	1.12	1.20	
"	12.50 "	6.3	1.07	1.22	1.20	1.20	1.30	1.20	1.20	
"	12.50 "	7.5	1.75		2.10		1.90		2.05	2.05
Dog Serum	5.0 cc									
+ Heparin	12.5 mg	7.6	1.75		0.08		0.10		0.09	0.10
Dog Serum	5.0 cc	7.6	1.75		0.03		0.00		0.00	0.00
" "	2.0 "	7.6	1.35						0.00	0.03
" "	0.4 "	7.6	1.35						0.66	0.60
Fastusol	12.5 mg	7.5	0.95	1.15	1.40	1.07	1.08		1.08	

* Tryptic activity expressed as Anson-Mirsky Units $\times 10^4$.
† pH controlled with phosphate buffer.

tested in the same way so that the effects of a known inhibitor could be seen. Mixtures of trypsin with serum plus heparin were also studied to determine whether or not heparin augments the inhibition due to serum alone. The apparent increase in tryptic activity seen following the addition of heparin or fastusol is unexplained, but is thought to be due to an action of these agents on the hemoglobin substrate. It was not due to the addition of color-producing substances since blank determinations have taken this into consideration.

In view of our inability to confirm the observation that heparin can inhibit to any substantial degree the proteolytic action of trypsin *in vitro*, it was decided to determine whether heparin has a prophylactic value against the injection of trypsin into any other animal than the rat, in which its antagonistic action to trypsin was quite marked. It was found that it has a similar prophylactic value in guinea pigs. Injection of varied doses of a trypsin preparation (Difco) into 33 normal animals indicated that the LD_{50} was approximately 66 mg per kilo and that 80 mg per kilo would kill 100% of animals. In 30 animals injected with heparin (45 to 130 mg per kilo) 10 minutes previously, the indicated LD_{50} of the same trypsin preparation was 114 mg per kilo and the 100% lethal dose approximately 200 mg per kilo. The results indicate an effect comparable to that seen in the rat.

If the protective effect of heparin is not due to a specific antitryptic action the possibility that it might be due to its anti-coagulant action suggests itself. Accordingly, the prophylactic value of another anti-coagulant (Fastusol) was examined. It was found that Fastusol in doses of 24 to 90 mg per kilo raised the LD_{50} of a trypsin preparation in rats from 140 mg per kilo to 250 mg per kilo. As Fastusol did not show marked anti-tryptic action when tested by the Anson-Mirsky procedure (see Table I), these results support the view that the anticoagulant action is important. Additional support for this is provided in that postmortem examination of both guinea pigs and rats dying from trypsin injections showed some hemorrhagic infarction of the lungs and a dilated right heart. These findings indicate that intravascular clotting with pulmonary embolism may be a factor in the lethal action of trypsin in these animals, even though no gross evidence of intravascular clotting was found. As it is known that the clotting action of trypsin on blood can be prevented by heparin,[10] the prophylactic value of heparin in these cases can be explained without requiring that heparin have a specific antitryptic action.

As yet no explanation for the effect of heparin in inhibiting the release of histamine from rabbit blood cells by trypsin has been offered.

Summary. Heparin does not appear to be a specific antitryptic substance since it prevents neither the fall in blood pressure pro-

[10] Ferguson, J. H., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 33.

duced by trypsin in the dog nor the proteolytic activity of trypsin on a hemoglobin substrate. However, heparin does protect guinea pigs against the lethal effects of intravenously injected trypsin.

The observation that fastusol, like heparin, protects rats against the lethal effects of trypsin is compatible with the notion that such a protective action on the part of heparin is related to its anticoagulant properties.

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"Lipid-Steroid" Fractions of Mouse Adrenal Lipids.

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The amount of the lipid material of the mouse adrenal gland was demonstrated by Vicari[1] to vary with the strain used. The technic and method applied was histological in nature. This report deals with initial experiments in separating the lipid material of adrenal glands of different strains of mice into 2 fractions. The purpose is to see wherein lies the true difference between strains, that is, whether the variations hold true for both fractions. The 2 fractions are designated "lipid," materials soluble in petroleum ether. These include cholesterol and cholesterol esters. The other fraction is "steroid" material not soluble in petroleum ether.

Material. Three inbred strains of mice were used. The histories of these are as follows: *C57black,* inbred for over 50 generations with a 1% incidence of mammary gland tumor in breeding females, and a 0% incidence in virgin females; *dba,* inbred for over 50 generations with a 70% incidence of mammary gland tumor in breeding females and a 50% incidence in virgin females; *ce* strain, inbred for 35 generations with less than 1% incidence of mammary gland tumor in breeding females and none in virgins. The animals used for these experiments were normal virgin females 49 days old. Tumors first appear in the tumor strains at 6 months old for breeding females and older for virgin females. The average individual body weight was 19.5 g. None of the animals used had tumors.

Procedure.† For each test 4 adrenals from 2 littermates were used. Two tests were made for each group. The glands were trimmed, weighed, homogenized with a 1:3 mixture of absolute alcohol and acetone A.R. The homogenate was refluxed for 12 hours on a steam bath. The petroleum extract yielded the "lipid" fraction while the 70% alcohol extract yielded the "steroid" fraction. Each extract was evaporated to dryness on a steam bath and the residue taken up in a 1:3 alcohol-acetone mixture and appropriate aliquots taken for analysis by the Van Slyke manometric method[2] for determination of carbon.

Results. Per adrenal gland the *C57black* strain has the highest amount of total carbon, "lipid" and "steroid" fraction, 0.73 mg, 0.61 mg, 0.12 mg respectively, while the *dba* strain, the lowest amount, 0.37 mg, 0.36 mg, 0.01 mg respectively. The *ce* strain has values in

TABLE I.
"Lipid" and "Steroid" Fractions in Adrenal Glands of Mice.

Amount of Carbon per mg of Adrenal Gland			
Strain	*C57 black*	*ce*	*dba*
	mg	mg	mg
Avg gld. wt.	2.75	4.02	2.80
	mg	mg	mg
Total carbon	0.26	0.15	0.133
"Lipid" Fr.	0.22	0.12	0.129
"Steroid" Fr.	0.04	0.03	0.003

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† This procedure was devised by Dr. K. Dobriner of the Memorial Hospital, New York City. We are deeply indebted for his assistance and suggestions.

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