ANAPHYLAXIS IN CHOPPED GUINEA PIG LUNG

II. Enhancement of the Anaphylactic Release of Histamine and Slow Reacting Substance by Certain Dibasic Aliphatic Acids and Inhibition by Monobasic Fatty Acids

By K. F. AUSTEN,*· ‡ M.D., and W. E. BROCKLEHURST,§ Ph.D.

(From the National Institute for Medical Research, London)

Although several workers have produced *in vitro* inhibition of the anaphylactic reaction with metabolic inhibitors (1, 2) and we have observed inhibition with chymotrypsin substrates and inhibitors (3), enhancement of the *in vitro* anaphylactic reaction has not been previously reported.

While evaluating ε-amino caproic acid, an inhibitor of plasminogen activation (4, 5), for its effect on the anaphylactic reaction in chopped guinea pig lung, we found that caproic acid (hexanoic) was a more potent inhibitor than ε-amino caproic acid. It was further observed that the ability of the unsubstituted fatty acids to inhibit the anaphylactic release of histamine increased with increasing chain length from valeric to dodecanoic acid. Substitution of the fatty acid with an alpha amino group completely abolished the inhibitory potential. This led to a study of other structurally related compounds, and the discovery that the four carbon dibasic aliphatic acids, succinic and maleic (*cis*-butenedioic), greatly enhance the anaphylactic release of histamine and slow reacting substance SRS-A. Other four carbon dibasic acids, such as malic and fumaric (*trans*-butenedioic), and several dibasic acids of greater or lesser chain length were without effect.

The inhibition produced by the monobasic fatty acids can be reversed by the enhancing effect of succinic acid and *vice versa*. Thus, these data show that compounds normally present in mammalian tissue can greatly influence the intensity of the *in vitro* anaphylactic reaction in chopped guinea pig lung.

Materials and Methods

Guinea pigs were actively sensitized with ovalbumin as previously described (3). Passively sensitized animals were obtained by the intrathoracic injection of serum containing 5 to 8 mg. of rabbit anti-human gamma globulin. The experimental procedure used to obtain the perfused, chopped guinea pig lung tissue and to study the effect of the aliphatic acids on anaphy-

---

* United States Public Health Service Postdoctoral Research Fellow.

‡ Present address: Massachusetts General Hospital, Boston.

§ Present address: Department of Pharmacology, University New Buildings, Edinburgh.

laxis was identical with that described in the preceding paper (3). The biological assay for
SRS-A was performed on the terminal ileum of the guinea pig in the presence of 5×10^{-7} M
atropine and 10^{-6} M mepyramine as reported by Brocklehurst (6). The values for SRS-A are
expressed in arbitrary units per gram wet lung tissue. A unit refers to $\frac{1}{20}$ ml. of a standard
sample of freeze-dried perfusate (6). The standard sample was obtained by pooling the per-
fusates obtained during *in vitro* anaphylaxis of sensitized guinea pig lungs.

The fatty acids were obtained mainly from British Drug Houses Ltd., Dorset, England,
and other commercial sources; analar succinic acid and maleic acid were supplied by Hopkin
and Williams Ltd., Essex, England; caproic acid was obtained from California Biochemical,
Los Angeles; undecanoic was supplied by Eastman Organic Chemicals, Rochester, New York;
L-leucine and α-keto glutaric acid were obtained from Roche Products Ltd., London, and
L-glutamic acid and DL-norleucine were obtained from L. Light & Co., Ltd., Bucks, England.
Epsilon amino caproic acid was obtained from Dr. J. M. Reugsegger of Lederle Laboratories,
Pearl River, New York, and Δ^2-hexenoic acid, heptanoic acid, octanoic acid, nonanoic acid,
and dodecanoic acid were provided by Dr. A. T. James. The human gamma globulin used as
antigen in some studies was obtained from the Lister Institute and was dialyzed against
several changes of 0.15 M saline prior to use.

Acids containing eight carbon atoms or less were generally dissolved directly in normal
Tyrode's solution. Nonanoic, decanoic, undecanoic, and dodecanoic are sparingly soluble in
water and were therefore converted to their potassium salts as suggested by Goodman (7)
prior to use. A weighed sample of fatty acid was dissolved in absolute ethanol and neutralized
with KOH using a pH meter. The ethanol was then evaporated off under vacuum and the salt
was dissolved in a measured volume of freshly boiled distilled water. This was then mixed with
an equal volume of two times normal strength Tyrode's solution so that the final solution was
normal Tyrode's containing the salt of the fatty acid. When these acids were compared with
those of shorter chain length, all the test solutions were prepared in this way.

RESULTS

Enhancement of the Anaphylactic Release of Histamine and SRS-A by Succinic Acid.
—In 20 consecutive experiments with different tissue samples the addition of 0.5 mM
succinic acid to the reaction mixture almost doubled the anaphylactic release of hista-
mine. The percentage of total tissue histamine released by antigen increased from an
average of 19.5 per cent to an average of 37.0 per cent when 0.5 mM succinic acid was
present. The results of the individual experiments are shown in Fig. 1. The per cent
of the total tissue histamine released by antigen from tissue samples in normal Ty-
rode's solution is plotted on the ordinate, and the increment in the per cent of total
tissue histamine released by antigen from samples of the same tissue in the presence
of 0.5 mM succinic acid is plotted on the abscissa. In general, the greater the anaphy-
lactic release of histamine by antigen alone, the greater the increment in the presence
of succinic acid. Succinic acid alone did not release histamine.

The effect of different concentrations of succinic acid on the anaphylactic release
of histamine and SRS-A is depicted in Fig. 2. Enhancement of the anaphylactic release
of both histamine and SRS-A was definite with as little as 0.05 mM succinic acid. Five
or 20 mM of succinic acid did not give significantly more enhancement than 0.5 mM,
and the latter concentration was used in most experiments. The suggestion of a sig-
moid dose-response line in Fig. 2 is also consistent with the results obtained on two
other occasions.

Effect of Variation in Preincubation Time on the Succinic Acid-Enhanced Anaphy-
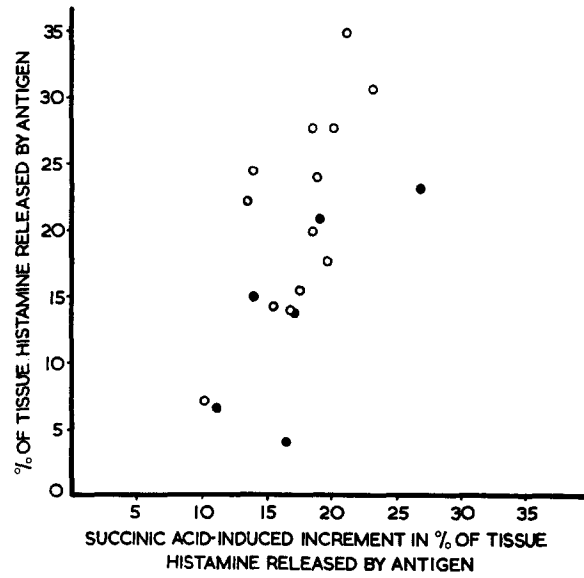
FIG. 1. Effect of succinic acid on the anaphylactic release of histamine. Antigen added 10 seconds after 0.5 mM succinic acid. The open circles refer to tissue from actively sensitized animals and the filled circles to tissue from animals passively sensitized with either rabbit anti-ovalbumin or rabbit anti-human gamma globulin.
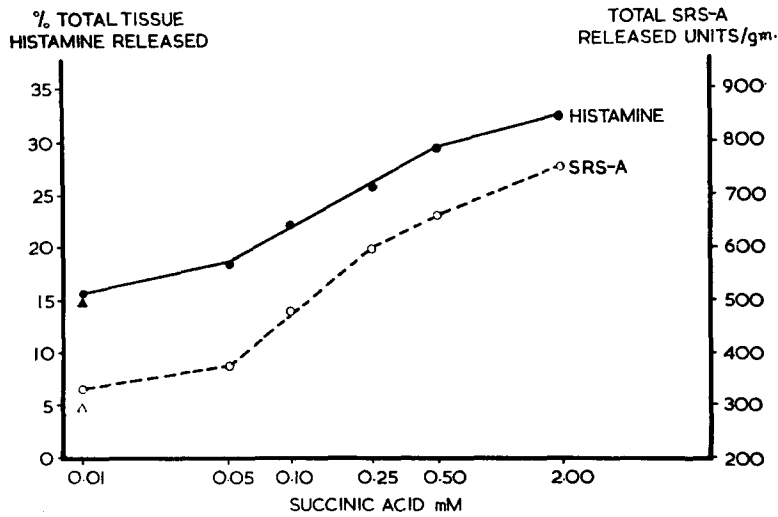

FIG. 2. Dose response curve for the effect of succinic acid on the anaphylactic release of histamine and slow reacting substance. The solid and open triangles respectively indicate the anaphylactic release of histamine and SRS-A from control tissue incubated in normal Tyrode's solution.

lactic Release of Histamine and SRS-A.—The effect of preincubating tissue for various time intervals with 0.1 and 1.0 mM succinic acid on the per cent of total tissue histamine released by antigen is shown in Fig. 3. At both concentrations incubation of the
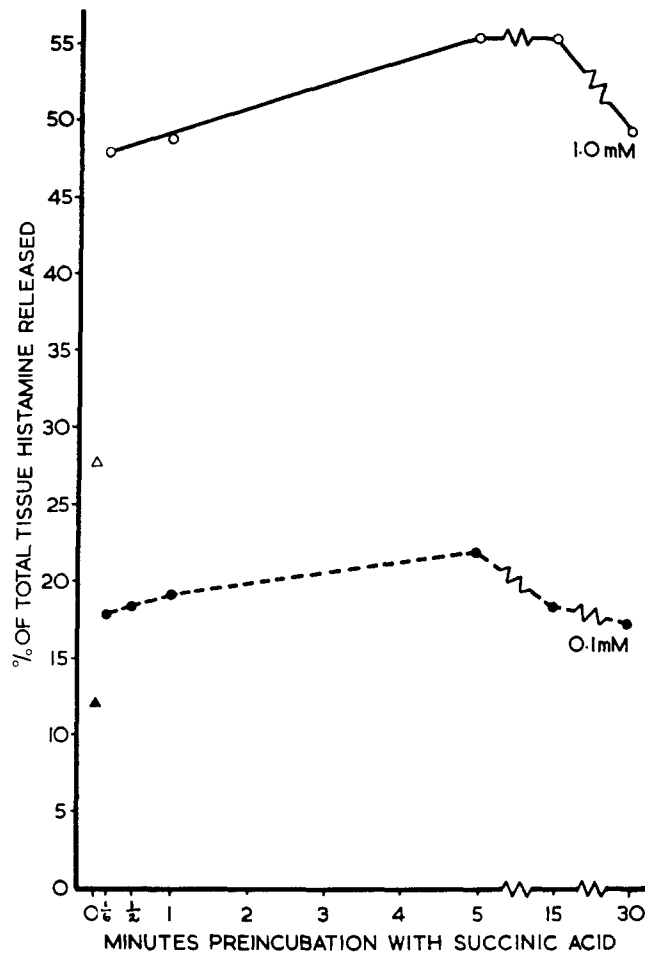

FIG. 3. Effect of variation in preincubation time on the succinic acid enchanced anaphylactic release of histamine. The open and solid triangles refer to the anaphylactic release of histamine from control tissue incubated in normal Tyrode's solution.

tissue with succinic acid for 5 minutes prior to adding antigen produced only about one-third more enhancement than the 10 second preincubation period. Thus, the 10 second preincubation period was used for all other experiments. When 0.1 mM succinic acid was used the enhancing capacity began to fall off after 5 minutes' preincubation, whereas with 1.0 mM it did not begin to decline until after 15 minutes. Succinic acid even produced enhancement when the antigen was added 10 seconds before the acid,

but the enhancement produced was only about half that obtained when the acid was added first.

Effect of Succinic Acid on the Time Course of Histamine and SRS-A Release.—Fig. 4 shows the time course of the release of histamine and SRS-A by antigen in the presence of 0.5 mM succinic acid. In this experiment the amounts released in 15 minutes in the presence of succinic acid were virtually twice those obtained in Tyrode's solution
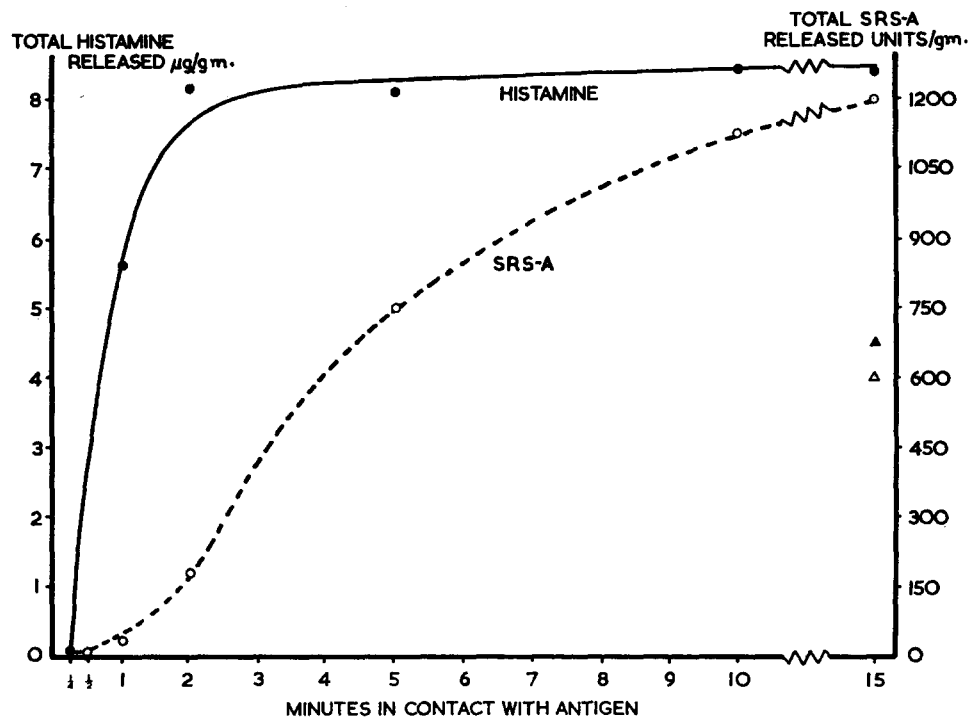

FIG. 4. Effect of 0.5 mM succinic acid on the time course of histamine and SRS-A release after addition of antigen. The solid and open triangles respectively indicate the anaphylactic release of histamine and SRS-A from control tissue incubated in normal Tyrode's solution.

alone. Histamine release began 10 to 15 seconds after antigen addition, and two-thirds of the total amount to be released could be detected by the end of the 1st minute. Thus, the time course for the anaphylactic release of histamine from chopped guinea pig lung in the presence of succinic acid is virtually identical with that observed in normal Tyrode's solution (3). SRS-A began to appear within 15 to 30 seconds after antigen addition but did not attain maximum release rate until after 2 minutes. The time course of SRS-A release is slower than that of histamine, as has been previously shown in perfusion experiments (6).

The Effect of Other Dibasic Aliphatic Acids on the Anaphylactic Release of Histamine and SRS-A.—The effect of certain dibasic acids and Krebs cycle intermediates on the

TABLE I

The Effect of Krebs Cycle Intermediates and Dibasic Acids on the Anaphylactic Release of Histamine from Chopped Guinea Pig Lung

Compound	Concentration	Enhancement in histamine release relative to that produced by 0.5 mM succinic acid*
	mM	
Succinic acid	0.5	1.00
Phenylsuccinic acid	5.0	0‡
	20.0	0
Citric acid	0.5	0
α-Ketoglutaric acid	0.5	0.12
	5.0	0.93
Fumaric acid	0.5	0
	5.0	0
	20.0	0
Malic acid	0.5	0
	5.0	0
Malonic acid	0.5	0.12
	5.0	0.22
	20.0	0.07
Glutaric acid	0.5	0.32
	2.5	0.66
	5.0	0.80
Adipic acid	0.5	0.16
	5.0	0.23
	20.0	0
Pimelic acid	0.5	0.13
Maleic acid	0.5	1.00
	5.0	1.28
	10.0	1.17

* The increase in the anaphylactic release of histamine attributable to the presence of 0.5 mM succinic acid was arbitrarily assigned a value of one, and the enhancement produced by the other compounds used in the same experiment is expressed relative to that produced by 0.5 mM succinic acid.

‡ Zero can be due to either absence of enhancement or minimal inhibition (see text).

anaphylactic release of histamine is shown in Table I. The increase in the anaphylactic release of histamine attributable to the presence of 0.5 mM succinic acid was arbitrarily
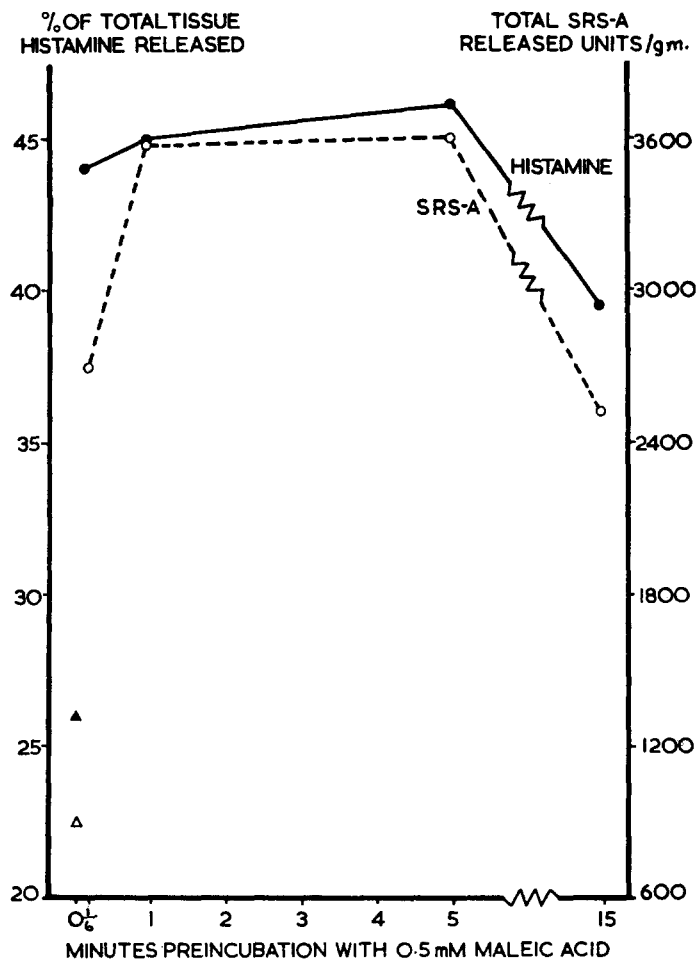

Fig. 5. Effect of variation in preincubation time on the maleic acid enhanced anaphylactic release of histamine and SRS-A. The concentration of maleic acid was 0.5 mM. The solid and open triangles respectively indicate the anaphylactic release of histamine and SRS-A from control tissue incubated in normal Tyrode's solution.

assigned a value of one, and the enhancement produced by the other compounds used in the same experiment is expressed relative to that produced by 0.5 mM succinic acid. The absolute increase in the anaphylactic release of histamine due to the presence of succinic acid in these experiments was never less than 67 per cent and in most instances exceeded 100 per cent.

The Krebs cycle intermediates fumaric and malic acid gave no enhancement at a final concentration of 0.5 or 5.0 mM, and fumaric acid was even slightly inhibitory at 20 mM. α-Keto glutaric acid, on the other hand, was capable of enhancing the anaphylactic release of histamine, but was only one-tenth as potent as succinic acid, in that 5.0 mM of α-keto glutaric acid was needed to achieve the same enhancement as 0.5 mM succinic acid. Citric acid did not enhance at 0.5 mM and was not studied at a higher concentration because calcium binding would have made it difficult to interpret the findings. The three, six, and seven carbon dibasic acids malonic, adipic, and pimelic respectively were virtually inactive, while glutaric had approximately the same activity as α-keto glutaric. Phenylsuccinic acid gave no enhancement at 5.0 mM and was slightly inhibitory at 20 mM.

TABLE II

*The Effect of Certain Aliphatic Acids on the Anaphylactic Release of Histamine and SRS-A from Passively Sensitized Guinea Pig Lung**

Compound	Concentra-tion	Histamine release	Enhancement (+) or inhibition (−)	SRS-A release	Enhancement (+) or inhibition (−)
	mM	*μg./gm.‡*	*per cent*	*units/gm.‡*	*per cent*
Control (Tyrode's solution)		4.4		225	
α-Ketoglutaric acid	0.5	5.1	+16	375	+66
Succinic acid	0.5	8.4	+91	750	+233
Fumaric acid	0.5	5.0	+14	300	+33
Maleic acid	0.5	8.5	+93	750	+233
Caproic acid	20.0	1.5	−66	45	−80

* Guinea pigs sensitized by the intrathoracic injection of 8 mg. rabbit anti-human gamma globulin.

‡ Release expressed per gram wet lung tissue.

In order to determine whether the spatial arrangement of the carboxyl groups of succinic acid (butanedioic acid) was important, the *cis* and *trans* isomers of butenedioic acid, maleic acid (*cis*-butenedioic) and fumaric acid (*trans*-butenedioic), were compared. Whereas fumaric was not active, maleic acid, the *cis* isomer, was found to be at least as active as succinic acid when compared at 0.5 mM (Table I).

The effect of preincubating tissue for various time intervals with 0.5 mM maleic acid on the anaphylactic release of histamine and SRS-A is shown in Fig. 5. Virtually maximum enhancement of histamine release was apparent after only 10 seconds' preincubation, and maximum SRS-A release was achieved after only 1 minute preincubation. In both instances the enhancement fell off after 5 minutes of preincubation.

Studies with Tissue from Passively Sensitized Animals.—In order to ascertain whether or not these observations on the enhancement of the anaphylactic reaction applied to other antigen-antibody systems and to passively sensitized tissue, studies were carried out with tissue from guinea pigs passively sensitized with rabbit anti-human gamma globulin. As shown in Table II, at a concentration of 0.5 mM succinic acid and maleic acid produced almost a 100 per cent increase in histamine release and

better than a 200 per cent increase in SRS-A release. At the same concentration α-keto glutaric acid and fumaric acid had a much smaller effect.

Inhibition of the Anaphylactic Release of Histamine by Monobasic Fatty Acids.—The dose response curve for the inhibition of the anaphylactic release of histamine from chopped guinea pig lung by caproic and decanoic acid is depicted in Fig. 6. By interpolation it can be seen that the concentration of caproic acid required to produce 50 per cent inhibition is 4.2 mM whereas only 0.58 mM of decanoic acid is required to produce the same inhibition. The addition of calcium chloride in excess of the caproic acid concentration on a molar basis does not reverse the inhibition; thus, the inhibition
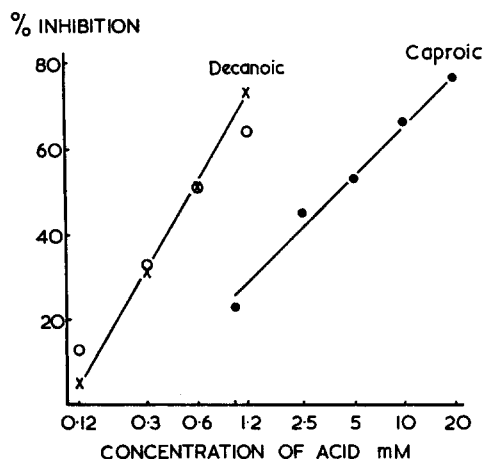

FIG. 6. Dose response curve for the inhibition of the anaphylactic release of histamine by caproic (hexanoic) and decanoic acid. The open circles refer to an experiment with decanoic acid in calcium and magnesium-free Tyrode's solution instead of normal Tyrode's.

cannot be attributed to binding of essential calcium by the fatty acid. The fact that calcium is essential for the anaphylactic release of histamine in chopped guinea pig lung was observed by Mongar and Schild (8).

The finding that decanoic acid was a more potent inhibitor than caproic acid prompted us to study the straight chain fatty acids from two to twelve carbons in length. Since the longer ones, undecanoic and dodecanoic, produced visible flocculation when their aqueous solutions were added to an equal volume of double strength Tyrode's solution, suggesting formation of calcium soaps, it was necessary to work with a calcium and magnesium free medium. In evaluating the feasibility of working with such a medium it was first essential to ascertain whether or not reproducible experiments were possible, and then to determine if the concentration of decanoic acid required to produce 50 per cent inhibition differed in normal Tyrode's and calcium-magnesium-free Tyrode's. The effect of incubating tissue for various intervals in a calcium-magnesium free medium prior to adding antigen is presented in Fig. 7. When the antigen was added just 10 seconds after the suspending medium had been changed from normal Tyrode's to calcium-magnesium free Tyrode's, the histamine release was only 72

per cent of that obtained in normal Tyrode's solution. However, the progressive impairment of antigen-induced histamine release after the first 10 seconds in the calcium-magnesium-free medium was much more gradual, so that the inhibition after 1 minute contact between the tissue and the modified medium was only slightly greater than that after just 10 seconds contact. Therefore, in all experiments using this calcium-magnesium-free medium the antigen was added 15 seconds after changing the suspending medium from normal Tyrode's to calcium-magnesium-free Tyrode's with or without the potential inhibitor.

Fig. 8 shows that the concentration of the fatty acids required to produce 50 per cent inhibition of histamine release decreases with increasing chain length from valeric to dodecanoic acid. Acetic, propionic, and butyric acid were not significantly inhibitory

HISTAMINE RELEASE
AS % OF THAT IN
NORMAL TYRODE
  SOLUTION

MINUTES TISSUE BATHED IN Ca^{++}& Mg^{++}FREE TYRODE SOL'N.
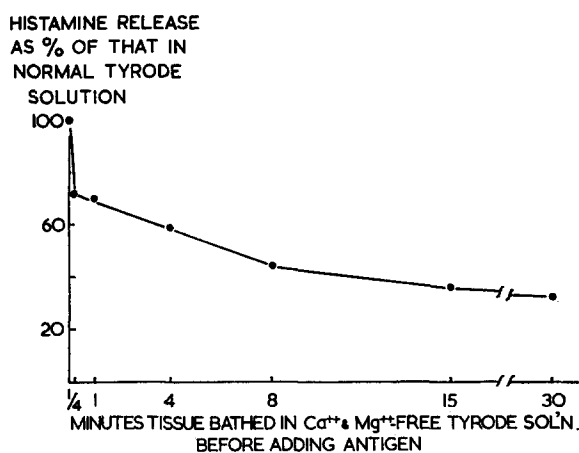BEFORE ADDING ANTIGEN

FIG. 7. The effect of preincubation in a calcium and magnesium-free medium on the anaphylactic release of histamine. After antigen addition the reaction was allowed to continue for 15 minutes at 37°C. in the usual way.

at 20 mM. The fact that the slope of the curve shown in Fig. 8 is steeper over the range of the shorter acids than over the range of the longer ones could mean that the effective concentration of the longer ones is diminished by calcium binding. However, as shown in Fig. 6 the concentration of decanoic acid required to produce 50 per cent inhibition of histamine release is virtually the same in calcium-magnesium-free Tyrode's as in normal Tyrode's solution. In other experiments in which the inhibitory effects of 1.0 mM octanoic, 0.75 mM nonanoic, and 0.5 mM decanoic acid were studied in normal and calcium-magnesium-free Tyrode's there was no significant difference. Thus, the shape of the plot in Fig. 8 is not readily explained by calcium binding.

In order to be certain that the fatty acid inhibitors did not destroy or release antibody or otherwise impair the ability of the sensitized tissue to respond to antigen, sensitized tissue was incubated with 1 mM decanoic acid for 15 minutes and then washed three times with Tyrode's solution before adding antigen. On the addition of antigen the tissue exhibited virtually the same histamine release as control tissue treated in a similar fashion with normal Tyrode's. When the tissue, in which the antigen-induced histamine release had been inhibited by the presence of decanoic acid,

CONCENTRATION
GIVING 50%
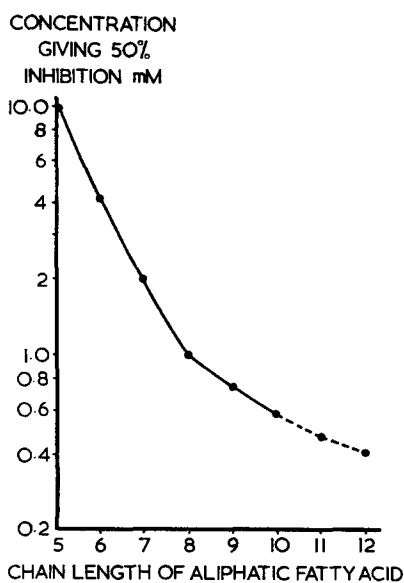INHIBITION mM

CHAIN LENGTH OF ALIPHATIC FATTY ACID

FIG. 8. Effect of increasing fatty acid chain length on the concentration required to produce 50 per cent inhibition of the anaphylactic release of histamine. The values were obtained either by interpolation from a dose response curve or by repeated experimental observation; the values for undecanoic and dodecanoic acid were obtained in calcium and magnesium free Tyrode's solution.

TABLE III

The Effect of Caproic Acid Analogs on the Anaphylactic Release of Histamine from Guinea Pig Lung

Compound	Inhibition*
	per cent
Caproic acid	85
Isocaproic acid	85
ε-Amino caproic acid	44
α-Amino caproic acid (DL-norleucine)	0
N-acetyl DL-norleucine	0
α,ε-diamino caproic acid (L-lysine)	0
N-acetyl-L-lysine	0
α-amino isocaproic acid (L-leucine)	0
Δ^2-Hexenoic acid	85
Adipic acid	0

* Per cent decrease in the antigen-induced release of histamine due to the presence of 20 mM of potential inhibitor in the reaction mixture.

was washed and treated a second time with antigen, there was no histamine release. Thus, decanoic acid does not prevent effective antigen-antibody interaction with resultant desensitization, and inhibits by acting on a reaction essential to the anaphylactic release of histamine.

Since fatty acids bind strongly to human serum albumin (7) and by so doing can alter the physiochemical characteristics of the albumin (9), it seemed possible that the fatty acid inhibition was due to binding and modification of the antigen, ovalbumin, by the fatty acid. For this reason experiments were carried out with an increasing ovalbumin concentration and with an entirely different antigen-antibody system. Increasing the concentration of the antigen, ovalbumin, 10- or even 50-fold produced no detectable reversal of the inhibition produced by 0.5 mM decanoic acid. Furthermore, as shown in Table II, caproic acid inhibited the anaphylactic release of histamine and SRS-A from tissue sensitized with rabbit anti-human gamma globulin.

The Effect of Analogs of Caproic Acid on the Anaphylactic Release of Histamine.—As shown in Table III, the introduction of an epsilon amino group diminished the inhibitory capacity of caproic acid by half, while the presence of an alpha amino group as in

TABLE IV

The Effect of Succinic Acid on the Inhibition of the Anaphylactic Release of Histamine and SRS-A Produced by Caproic Acid

Reaction mixture		Histamine release	Enhancement (+) or inhibition (−)	SRS-A release	Enhancement (+) or inhibition (−)
Succinic	Caproic				
mM	*mM*	*µg./gm.*￼*	*per cent*	*units/gm.*￼*	*per cent*
		9.2‡		1050‡	
0.5		16.2	+76	2100	+100
	5.0	5.4	−41	630	−40
0.5	5.0	13.0	+41	1580	+50
	20.0	3.7	−60	210	−80
0.5	20.0	7.2	−22	630	−40

* Release expressed per gram wet lung tissue.

‡ Control values obtained by the anaphylactic release of histamine and SRS-A in normal Tyrode's solution.

DL-norleucine or L-lysine, abolished this activity completely. Similarly, isocaproic acid was as potent an inhibitor as caproic acid, but the corresponding alpha amino acid, L-leucine, was without effect. Acetylation of the alpha amino group as in N-acetyl-L-lysine and N-acetyl-DL-norleucine did not restore the inhibitory capacity (3). Δ^2-Hexenoic acid was as effective as caproic acid in inhibiting the anaphylactic release of histamine, but the dibasic six carbon acid, adipic, was without effect.

Effect of Succinic Acid on the Inhibition of the Anaphylactic Release of Histamine and SRS-A Produced by Caproic Acid or L-Tryptophan Ethyl Ester.—Since the monobasic fatty acids and certain dibasic aliphatic acids have opposite effects on the *in vitro* anaphylactic reaction, succinic acid was tested against two concentrations of caproic acid to see if there was evidence of competition or if one effect was predominant. Table IV shows that a concentration of 0.5 mM of succinic acid produces only partial reversal of the inhibition due to 20 mM of caproic acid but achieves complete reversal of the inhibition due to 5 mM caproic acid. In fact in the presence of 5 mM caproic acid the succinic acid produces enhancement. Similarly, 0.5 mM succinic acid achieved only

partial reversal of the inhibition due to 2 mM decanoic acid, but produced enhancement in the presence of 0.5 mM decanoic acid.

The ability of 0.5 mM succinic acid to reverse caproic acid inhibition and L-tryptophan ethyl ester inhibition (3) is compared in Table V. In the concentrations used, caproic acid and the ester each produced about 70 per cent inhibition of histamine release and over 80 per cent inhibition of SRS-A release, but the capacity of succinic acid to reverse these inhibitions was very different. The inhibition of histamine and SRS-A release due to the presence of 20 mM caproic acid was reduced from 72 and 87 per cent to 19 and 24 per cent respectively by the addition of 0.5 mM succinic acid, whereas that due to 2.5 mM L-tryptophan ethyl ester was only reduced from 68 and 81 per cent to 53 and 63 per cent respectively. When 5 mM of the ester was used 0.5 mM succinic acid gave virtually no reversal of histamine and SRS-A inhibition.

TABLE V

The Effect of Succinic Acid on the Inhibition of the Anaphylactic Release of Histamine and SRS-A Produced by Caproic Acid and L-Tryptophan Ethyl Ester (TRYEe)

Reaction mixture			Histamine release	Enhancement (+) or inhibition (−)	SRS-A release	Enhancement (+) or inhibition (−)
Succinic	Caproic	TRYEe				
mM	mM	mM	μg./gm.*	per cent	units/gm.*	per cent
			8.1‡		1050‡	
0.5			12.8	+58	2100	+100
	20.0		2.3	−72	135	−87
0.5	20.0		6.6	−19	800	−24
		2.5	2.6	−68	195	−81
0.5		2.5	3.8	−53	390	−63

* Release expressed per gram wet lung tissue.

‡ Control values obtained by the anaphylactic release of histamine and SRS-A in normal Tyrode's solution.

DISCUSSION

The observation that the *in vitro* anaphylactic reaction in chopped guinea pig lung can be consistently enhanced by succinic and maleic acid, which by themselves do not release histamine and SRS-A, indicates that these acids are probably acting on an antigen-antibody activated step. In general a concentration of 0.5 mM of succinic or maleic acid doubles the absolute anaphylactic release of histamine or SRS-A. Enhancement is definite with as little as 0.05 mM succinic acid (Fig. 2) and increasing the concentration to 5 or 20 mM does not give significantly more enhancement than is obtained with 0.5 mM. Prolonged preincubation of the tissue with the enhancing acids is not required (Fig. 3 and 5). 60 to 90 per cent of the maximal enhancement achieved is obtained when the antigen is added to the tissue only 10 seconds after succinic or maleic acid.

The time course of histamine release in the presence of succinic acid (Fig. 4) is virtually identical with that observed in normal Tyrode's solution (3). In

both instances release begins within 10 to 15 seconds of adding antigen and two-thirds of the total histamine to be released is detectable within 1 minute. Thus, the enhancement is due to a quantitative increase in the release during the 1st minute and not to a prolongation of the period of maximal release. The increased release in the presence of succinic and maleic acid is not due to increased histamine formation in the tissue. The sum of the histamine released and that left in the tissue after anaphylaxis is not greater than the histamine content of control tissue exposed to neither succinic acid nor antigen.

The succinic acid enhancement of the anaphylactic reaction cannot be attributed to an effect on the tricarboxylic acid cycle because other intermediates such as fumaric acid, malic acid, and citric acid are without effect. Furthermore, maleic acid, which is not a Krebs cycle intermediate, is as active as succinic acid in augmenting the *in vitro* anaphylactic reaction. The moderate activity exhibited by α-keto glutaric acid is due either to the fact that glutaric acid itself has moderate enhancing activity or due to partial conversion of the α-keto glutaric to succinic acid. The enhancement produced by succinic and maleic acid is probably due to potentiation of some step activated by the antigen-antibody interaction and common to both the release of histamine and SRS-A.

Studies in which succinic acid is compared with malonic, glutaric, adipic, and pimelic acid, which are three, five, six, and seven carbons in length respectively, reveal that the four carbon length is optimal (Table I). Substitution of succinic acid with a phenyl group (phenylsuccinic acid) or a hydroxyl group (malic acid) abolishes all enhancing activity. The *trans* isomer of butenedioic acid, fumaric acid, is not active, but the *cis* isomer, maleic acid, is as active as succinic. Thus, the structural configuration required for a dibasic acid to enhance the *in vitro* anaphylactic reaction is quite specific; the carboxyl groups should be separated by a 2 carbon chain, and must be free or fixed in the *cis* position.

The monobasic fatty acids from valeric to dodecanoic inhibit the anaphylactic release of histamine, and the concentration necessary to produce 50 per cent inhibition decreases with increasing chain length (Fig. 8). The fatty acids also inhibit the anaphylactic release of SRS-A (Tables IV and V). The introduction of a polar group, amino or carboxyl, into the hydrocarbon residue of caproic acid diminishes or abolishes inhibitory capacity, but unsaturation or terminal branching of the hydrocarbon chain does not decrease inhibitory activity (Table III).

The inhibition produced by the fatty acids is not due to binding of essential calcium (8), because calcium excess produces no detectable reversal. Since the fatty acids bind strongly to albumin (7) and by so doing can alter the physiochemical properties of albumin (9), it was possible that the fatty acids could inhibit anaphylaxis by modifying the antigen or by altering some essential

protein activated by the antigen-antibody interaction. This possibility is supported by the fact that the ability of the monobasic fatty acids to bind to albumin (7, 10) and to stabilize against denaturation by heat or urea (11, 12) increases with increasing chain length, and decreases with the introduction of an amino group into the hydrocarbon residue. On the other hand, the fact that increasing the concentration of antigen 10- or even 50-fold does not produce detectable reversal of inhibition, and the finding that caproic acid is as effective in inhibiting anaphylaxis when the antigen is human gamma globulin as when the antigen is ovalbumin, suggests that the inhibition is not due to binding and modification of the antigen. The failure of decanoic acid to inhibit if washed from the tissue prior to antigen addition shows that the antibody is intact. Furthermore, the observation that the inhibited tissue is desensitized to a second dose of antigen indicates that effective antigen-antibody interaction must have taken place in the presence of the decanoic acid. Thus, the monobasic fatty acids probably inhibit a reaction set off by the union of antigen and antibody, and essential to both the release of histamine and SRS-A.

Since both the inhibiting monobasic fatty acids and the enhancing dibasic aliphatic acids seem to be acting on an antigen-antibody activated step, it is possible that they are competing for the same site in the reaction sequence. The fact that 5 mM caproic acid and 0.5 mM decanoic acid produce only moderate reversal of succinic acid enhancement, while a concentration of the acids four times as great produces complete reversal (Table IV), does not necessarily imply competition for the same site, but does suggest that this may be the case.

Since a variety of chymotrypsin substrates and inhibitors have been shown to inhibit the anaphylactic release of histamine (3) the ability of succinic acid to reverse the effect of one of these inhibitors was studied. It was found that the inhibition produced by the chymotrypsin substrate, L-tryptophan ethyl ester, is more resistant to reversal by succinic acid than the inhibition due to caproic acid (Table V). This suggests that the ester and the acid inhibit the anaphylactic reaction in guinea pig lung at different steps in the reaction sequence, or at least in a different way.

Some of the substances shown to have inhibiting or enhancing activity are naturally occurring and present in mammalian tissue. The role of such substances in determining the severity of the *in vivo* anaphylactic reaction may be of importance, in view of the low concentrations which are effective *in vitro* in the guinea pig.

SUMMARY

The quantitative release of histamine and slow reacting substance by specific antigen from perfused, chopped, sensitized guinea pig lung has been used to study the opposing effects of monobasic and certain dibasic fatty acids on the anaphylactic reaction.

The anaphylactic release of histamine and slow reacting substance is doubled by the addition of 0.5 mM of succinic or maleic acid to the reaction mixture, and enhancement is definite with as little as 0.05 mM of succinic acid. Prolonged preincubation of the tissue with the dibasic acids is not required, for 60 to 90 per cent of maximal enhancement is apparent when the antigen is added to the tissue only 10 seconds after succinic or maleic acid. The increased histamine release is not due to a qualitative change in the time course of histamine release, is not the result of increased histamine formation, and cannot be attributed to an effect on the tricarboxylic acid cycle. The enhancement seems to be due to potentiation of some step activated by the antigen-antibody interaction and common to both the release of histamine and slow reacting substance. The structural configuration required for a dibasic acid to enhance the anaphylactic reaction in guinea pig lung is quite specific; the carboxyl groups should be separated by a two carbon chain, and must be free or fixed in the *cis* position.

The monobasic fatty acids from valeric to dodecanoic inhibit the anaphylactic release of histamine, and the concentration needed to produce 50 per cent inhibition decreases with increasing chain length. The introduction of a polar group, amino or carboxyl, into the hydrocarbon residue diminishes or abolishes inhibitory capacity. The inhibition produced by the fatty acids is neither due to calcium binding nor due to prevention of effective antigen-antibody interaction; the fatty acids probably inhibit by acting on an antigen-antibody-activated step.

The inhibition of the anaphylactic release of histamine and slow reacting substance produced by caproic or decanoic acid can be reversed by the enhancing effect of succinic acid, and *vice versa*. Thus, compounds normally present in mammalian tissue can greatly influence the intensity of the *in vitro* anaphylactic reaction in the guinea pig.

Since this manuscript was submitted for publication, it has been brought to our attention that Moussatche and Danon (13) observed that succinic acid enhanced the anaphylactic release of histamine; however, these workers attributed the enhancement to an effect on the tricarboxylic acid cycle and so did not study other Krebs cycle intermediates.

BIBLIOGRAPHY

1. Mongar, J. L., and Schild, H. O., Inhibition of the anaphylactic reaction, *J. Physiol.*, 1957, **135,** 301.
2. Chakravarty, N., The mechanism of histamine release in anaphylactic reaction in guinea pig and rat, *Acta Physiol. Scand.*, 1960, **48,** 146.
3. Austen, K. F., and Brocklehurst, W. E., Anaphylaxis in chopped guinea pig lung, I. Effect of peptidase substrates and inhibitors, *J. Exp. Med.*, 1961, **113,** 521.
4. British Patent Specification, 770,693, Her Majesty's Stationery Office, London, 1957.
5. Alkjaersig, N., Fletcher, A. P., and Sherry, S., ε-Aminocaproic acid: an inhibitor of plasminogen activation, *J. Biol. Chem.*, 1959, **234,** 832.

6. Brocklehurst, W. E., The release of histamine and formation of slow reacting substance (SRS-A) during anaphylactic shock, *J. Physiol.*, 1960, **151**, 416.

7. Goodman, D. S., The interaction of human serum albumin with long-chain fatty acid anions, *J. Am. Chem. Soc.*, 1958, **80**, 3892.

8. Mongar, J. L., and Schild, H. O., The effect of calcium and pH on the anaphylactic reaction, *J. Physiol.*, 1958, **140**, 272.

9. Schmid, K., Electrophoresis of human serum albumin at pH 4.0 I. A systematic study on the effect of organic acids and alcohols upon the electrophoretic behavior of albumin, *J. Biol. Chem.*, 1959, **234**, 3163.

10. Teresi, J. D., and Luck, J. M., The combination of organic anions with serum albumin VIII. Fatty acid salts, *J. Biol. Chem.*, 1952, **194**, 823.

11. Boyer, P. D., Lum, F. G., Ballou, G. A., Luck, J. M., and Rice, R. G., The combination of fatty acids and related compounds with serum albumin, I. Stabilization against heat denaturation, *J. Biol. Chem.*, 1946, **162**, 181.

12. Boyer, P. D., Ballou, G. A., and Luck, J. M., The combination of fatty acids and related compounds with serum albumin II. Stabilization against urea and guanidine denaturation, *J. Biol. Chem.*, 1946, **162**, 199.

13. Moussatche, H., and Danon, A. P., On the action of succinate and malonate upon the histamine released during the anaphylactic reaction *in vitro, Naturwissenschaften*, 1957, **44**, 330.