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# The Differentiation of the Chloroform, Peptone and Antigen-Antibody Inducible Esterase Activities of Human Serum from Plasmin*†

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**Summary.** A comparative study which differentiates the chloroform inducible esterase activity of human serum from plasmin is presented. Although both esterase activities have a similar pH optimum against p-toluene sulfonyl L-arginine methyl ester, they differ in, at least, six other respects. The proenzyme of the chloroform inducible esterase activity is destroyed by heating at 56° for 30 minutes or by reducing the pH to 2.3, whereas plasminogen is stable to such treatment. The chloroform inducible esterase activity is resistant to soybean trypsin inhibitor while plasmin is mostly inhibited. Activity against N-acetyl L-tyrosine ethyl ester is induced by chloroform, but plasmin is inactive against this substrate. On the other hand, plasmin splits casein but the chloroform preparation does not. Pretreatment of serum with an antigen-antibody precipitate greatly diminishes the chloroform inducible esterase activity but not the streptokinase inducible activity.

Evidence is presented which suggests that the chloroform inducible esterase activity consists of two components: one is predominantly active against p-toluene sulfonyl L-arginine methyl ester and is probably not affected by the immune precipitate; the other is more active against N-acetyl L-tyrosine ethyl ester than against p-toluene sulfonyl L-arginine methyl ester, is removed by exposing serum to immune precipitate and bears a significant resemblance to the activated first component of complement.

Peptone inducible esterase activity, like that induced by chloroform, arises from a heat and acid labile precursor, is active against p-toluene sulfonyl L-arginine methyl ester and N-acetyl L-tyrosine ethyl ester, is not inhibited by soybean trypsin inhibitor, does not digest casein and is partially removed by exposing serum to the immune precipitate before peptone activation.

The esterase activity taken up from serum by the immune precipitate, like that induced by chloroform or peptone, arises from a heat labile precursor, is active against p-toluene sulfonyl L-arginine methyl ester and N-acetyl L-tyrosine ethyl ester, is not inhibited by soybean trypsin inhibitor, and does not digest casein.

In view of the evidence that chloroform, peptone and antigen-antibody activate a similar, if not identical, proesterase, which is distinct from plasminogen, the possible role of this enzymic activity in histamine release is considered.

## INTRODUCTION

The fibrinolytic activity appearing in serum and plasma during the course of *in vivo* anaphylactic (Lowell, Franklin, Schiller and Follensby, 1956) and anaphylactoid (Rocha

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e Silva, Andrade and Teixeira, 1946) reactions and in a variety of disease states (Ratnoff, 1949; Meyers, Burdon and Riley, 1957; Cliffton, 1952; Scott, Matthews, Butterworth and Frommeyer, 1954) not associated with anaphylactic signs and symptoms has been attributed to a proteolytic enzyme, plasmin. The enormous literature which has grown up on the nature of this enzyme and on its possible implication in various physiologic and pathologic states has been reviewed by a number of workers (Macfarlane and Biggs, 1948; Astrup, 1956; Sherry, Fletcher and Alkjaersig, 1959). Plasmin normally exists in serum in an inactive precursor form, plasminogen. In human serum the precursor can be activated by streptokinase (SK)* alone (Christensen, 1945), whereas in a number of other species a 'proactivator', obtained from human serum, as well as SK is required (Norman, 1957; Meyers and Burdon, 1957). It is believed that SK reacts with the 'proactivator' to form an 'activator' which in turn converts plasminogen to plasmin (Norman, 1957; Meyers and Burdon, 1957). Troll, Sherry and Wachman (1954) have shown that human plasmin, like trypsin, is not only a protease capable of splitting conventional protein substrates, but also can act as an esterase capable of splitting synthetic substrates such as p-toluene sulfonyl L-arginine methyl ester (TAMe) and L-lysine ethyl ester. Moreover, it was subsequently demonstrated that the caseinolytic activity of human plasmin (Troll and Sherry, 1955) or guinea-pig plasmin (Meyers and Burdon, 1957) was competitively inhibited by the presence of these amino-acid esters.

Human serum can also be rendered proteolytic by chloroform treatment; this has been observed repeatedly by workers using clot lysis (Tagnon, Davidson and Taylor, 1942; Rocha e Silva and Rimington, 1948), caseinolysis (Ratnoff, 1948, a and b), and gelatin splitting (Christensen and MacLeod, 1945) as a measure of proteolytic activity. Geiger (1952), has shown that chloroform treated guinea-pig serum is fibrinogenolytic. Christensen and MacLeod (1945) using a gelatin digestion assay compared the chloroform-induced human protease with the SK activated protease and concluded that the two proteases were identical because of similarity in pH of optimum activity, pH of maximum stability and temperature-activity relationships. These same workers (Christensen and MacLeod, 1945; Christensen, 1946) suggested that the activation of plasminogen by SK was catalytic whereas activation by chloroform was auto-catalytic and initiated by the removal of an inhibitor. Subsequently other workers (Pillemer, Ratnoff, Blum and Lepow, 1953; Lepow, Wurz, Ratnoff and Pillemer, 1954) demonstrated that either chloroform or SK activation of serum protease was associated with the disappearance of the first (C′1), second (C′2) and fourth (C′4) components of complement from serum and postulated that the destruction of C′2 and C′4 was due to the activation of C′1 by the proteases.

Fibrinogenolytic (Ungar and Mist, 1949; Ungar, Damgaard and Hummel, 1953) and fibrinolytic (Olesen, 1957; Astrup and Olesen, 1957) activity can also be induced in guinea-pig serum by peptone treatment. Furthermore, the intravenous administration of peptone to dogs has been shown to result in fibrinolysis, histamine release, hypotension, leukopenia and thrombocytopenia (Rocha e Silva and Teixeira, 1946; Scroggie, Jacques and Rocha e Silva, 1947; Beraldo, 1950). The similarity of this reaction to anaphylaxis has prompted some workers to suggest that protease activation plays a role in histamine release in anaphylaxis (Ungar, 1956; Rocha e Silva, 1956).

* The following abbreviations will be used: streptokinase = SK; p-toluene sulfonyl L-arginine methyl ester = TAMe; benzoyl L-arginine methyl ester = BAMe; N-acetyl L-tyrosine ethyl ester = ATEe; soybean trypsin inhibitor = SBI; tris (hydroxymethyl) amino-methane buffer = tris; complement = C′; and the four components of complement = $C'_1$, $C'_2$, $C'_3$, and $C'_4$.

In this regard, Humphrey and Jaques (1955) have demonstrated an interesting difference between chloroform and peptone activated rabbit serum, on the one hand, and SK activated serum on the other. These workers observed that chloroform or peptone activated serum was capable of releasing histamine from rabbit platelets whereas SK activated rabbit or human serum was not. Because of this difference and the fact that esterase studies of chloroform and peptone activated human serum were not available, a comparative study of the chloroform, peptone, and SK induced esterase activity of human serum was undertaken. The present report is mostly concerned with the differentiation of the chloroform and SK inducible esterase activity of human serum by measurement of: (1) heat and acid stability of the proenzymes; (2) inhibition of the esterase activities by soybean trypsin inhibitor (SBI); (3) activity on casein; (4) substrate specificity with special reference to activity against N-acetyl L-tyrosine ethyl ester (ATEe); and (5) effect of an antigen-antibody complex on these esterase activities. Identical criteria were used to distinguish the peptone inducible esterase activity of human serum from plasmin, but most of these studies are not presented in detail.

The data obtained suggest that chloroform, peptone and antigen-antibody precipitate activate a similar, if not identical, proesterase and that this proesterase is distinct from plasminogen.

## MATERIALS AND METHODS

All experiments were performed with fresh, once frozen, non-pooled human serum obtained in 200 ml. lots from donors as needed. Five hundred ml. of blood were allowed to clot in the cold, the serum was removed and frozen in 5 ml. portions, and these were thawed just before use. All activation studies were carried out with serum or euglobulin prepared from serum rather than with some more refined product, so as to avoid, if possible, the loss of any unknown potential esterase system by purification. Chloroform* treated serum was prepared by adding 0.05 ml. chloroform per ml. serum, shaking vigorously in a thick-walled glass centrifuge tube by hand for 4 minutes, centrifuging in the cold for 30 minutes at 10,000 rev./min., and immediately removing the supernatant. Further removal of the chloroform from the supernatant by evaporation was not found to be necessary. Maximum chloroform activation was obtained by permitting the supernatant to stand at 4° for 4–7 days. Human euglobulin was prepared from plain or chloroform treated serum as follows: 1 ml. serum was diluted with 19 ml. distilled water in the cold, brought to pH 5.2 with cold 2 per cent acetic acid, allowed to stand in the cold for 15 minutes, and centrifuged 10 minutes in the cold at 2000 rev./min.; the precipitate was dissolved in 1.0 ml. of 0.15 M saline to give 1 : 1 euglobulin. Peptone activation was obtained by adding proteose peptone,† 50 mg. per ml. serum, incubating 5 minutes at 37°, and converting to euglobulin by dilution and acidification to pH 6.2. This procedure was necessary because peptone treated serum exhibited only low activity, while dilution and acidification to a pH lower than 5.8 resulted in complete loss of peptone induced activity; in peptone experiments the control euglobulin was also prepared at pH 6.2. Decalcified serum was obtained by treatment with Dowex-50, sodium form, resin. The effluent was kept at 4° during collection and was used immediately; calcium chloride was added for recalcification. Several pools of rabbit antiserum to bovine serum albumin were provided through the generosity of Dr. Sidney Leskowitz; the pools were

* Chloroform USP (Mallinkrodt).
† Difco proteose peptone.

obtained by injection of crystalline bovine serum albumin, emulsified in Freund's adjuvant into rabbits followed by intravenous booster injections. Streptokinase,* 20,000 units per ampule, was dissolved in 2.0 ml. distilled water before each experiment.

The substrates TAMe, BAMe and ATEe were obtained commercially;† ethyl cellusolve in a final concentration of 10 per cent in the reaction mixture was used to dissolve the ATEe. Casein‡ was prepared as outlined by Norman (1957). SBI§ was dissolved in four parts saline and one part 0.5 M tris buffer pH 7.5.

The esterase activity of serum was measured by a slight modification of the formol titration method of Troll, Sherry and Wachman (1954). After 5 minutes' preincubation at 37° to bring all reactants to a constant temperature, serum was added to substrate, saline and the appropriate tris buffer to give a final volume of 4.0 ml. After inversion of the mixture several times, a 1.0 ml. aliquot was removed for the zero time determination. The remaining 3.0 ml. were incubated at 37° and 1.0 ml. aliquots were removed at 25 and 45 minutes. In order to maintain linear activity with time, aliquots were taken at 10 and 20 minutes or 15 and 30 minutes when the serum preparation was particularly active. Each aliquot was added to 1.0 ml. of 37 per cent formaldehyde, pH 7.0–7.5. The amount of acid produced was then determined by measuring the amount of 0.06 N NaOH required to bring the reaction mixture to a pH of 7.2. The NaOH added was measured with a Scholander (1942) microburette, and the pH was determined by means of a Beckman Probe Electrode attached to a Beckman model G pH meter (Iselin and Niemann, 1950). A mechanical stirring device was used to obtain adequate mixing of the added NaOH. Serum activated with SK was incubated for 3 minutes at 37° before mixing with substrate. When the effect of SBI was studied the SK or chloroform activated serum was incubated with the inhibitor for 2 minutes before mixing with substrate.

In general, when plasmin was studied, the reaction mixture contained 0.8 ml. of 0.1 M substrate, 0.4 ml. of serum, 1.0 ml. of 0.5 M pH 8.3 tris buffer, 0.2 ml. of SK, and saline to bring to a volume of 4.0 ml. A concentration of 2000 units SK was selected because it consistently produced maximal activation. When chloroform or peptone activation was studied 0.8 ml. serum or the equivalent of euglobulin was used. Activity in all experiments has been expressed as micromoles of acid produced per 30 minutes per ml. serum or euglobin equivalent, and has been corrected by subtracting spontaneous breakdown of substrate and activity contained in the activating materials.

The casein assay was performed as described by Norman (1957) except that serum was used and the digestion time was increased to 60 minutes. When plasmin was studied the reaction mixture contained 0.5 ml. serum, 0.2 ml. SK, 1.0 ml. of 4 per cent casein, and saline to bring to 2.0 ml. When chloroform or peptone activation was investigated, 0.5 to 1.0 ml. serum or the equivalent of euglobulin was used. Activity has been expressed as the increase in optical density (280 mμ) per 60 minutes per 0.5 ml. serum, and has been corrected by subtracting the optical density of a control tube to which trichloroacetic acid was added at zero time.

In all experiments the antigen-antibody precipitate was formed at equivalence. The precipitate was washed twice in the cold with 0.15 M saline. Ice-cold human serum was added to the precipitate in the proportion of 0.15 to 0.30 mg. antibody nitrogen per ml. serum. The precipitate, suspended as finely as possible in serum, was allowed to stand in

* Varidase (Lederle).
† Amino-acid esters were obtained commercially from Mann Research Laboratories, Inc.
‡ Hammarsten casein.
§ Worthington Biochemical Corp.

the cold for ½ hour with occasional inversion of the tube. The mixture was then centrifuged for 30 minutes at 5000 rev./min. and the supernatant removed and studied for esterase activity. In those experiments in which the esterase content of the precipitate was also to be determined the precipitate was washed twice with 0.04 M saline (Lepow, Ratnoff and Pillemer, 1956), suspended in a finely divided form in 0.15 M saline, and assayed for esterase activity in the usual way. Elution of the precipitate as described by Lepow, *et al.* (1956) but without final dialysis yielded activity per ml. of original serum that was similar to that obtained by these workers; however, as the eluate contained only about 20 per cent as much activity as the suspension, the latter was used for most studies.

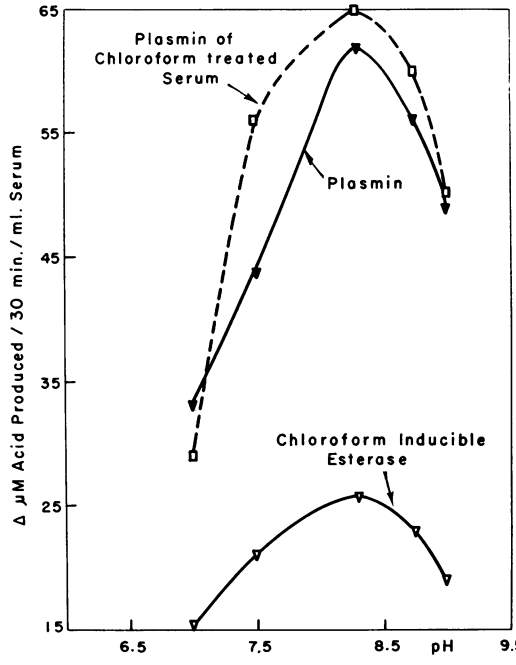


FIG. 1. *Effect of pH on the TAMe esterase activity of human serum.* The activity plotted is the increment due to the last step in the activation procedure (see text) when the final pH of the assay was varied as noted on the abscissa.

## RESULTS*

### pH ACTIVITY OPTIMUM OF THE SK AND CHLOROFORM INDUCIBLE TAMe ESTERASE ACTIVITY OF HUMAN SERUM

In Fig. 1 the pH activity curve of human serum activated with SK to form plasmin is compared with the pH activity curves obtained by chloroform activation, and by SK activation of serum pretreated with chloroform. The activity plotted is the net activity due to the last step in the activation procedure; thus, plasmin refers to the increment in TAMe esterase activity due to the addition of SK to serum, chloroform inducible esterase refers to the increment in activity due to the chloroform treatment of serum, and plasmin

* Although the data recorded in each table concern only a single experiment, each experiment presented is representative of several experiments giving similar results with serum from different donors. In addition, each experiment is designed so as to include its own controls.

of chloroform treated serum refers to the increment in activity due to the addition of SK to serum pretreated with chloroform. The pH optimum of the esterase activity induced by each of the three treatments was approximately 8.3. The preactivation of serum with chloroform did not diminish the subsequent increment from SK addition; indeed, the plasmin activity of serum pretreated with chloroform equalled or slightly exceeded that from untreated serum even though the former was corrected for the chloroform induced increment in esterase activity.

TABLE I
THE EFFECT OF CHLOROFORM ON HUMAN SERUM TAMe ESTERASE ACTIVITY

| *Experiment number* | *Ml. chloroform per ml. serum* | μM *acid produced per* 30 *min. per ml. serum* | |
|---|---|---|---|
| | | *Total activity* | *Increment due to activation** |
| 1 | 0 | 20 | |
| | 0.6 | 46 | 26 |
| | 0.3 | 46 | 26 |
| | 0.1 | 49 | 29 |
| 2 | 0 | 21 | |
| | 0.05 | 47 | 26 |
| | 0.03 | 48 | 27 |
| | 0.01 | 20 | 0 |

* Increment in activity obtained by chloroform treatment.

EFFECT OF CHLOROFORM CONCENTRATION AND TIME ON THE ACTIVATION OF THE CHLOROFORM INDUCIBLE TAMe ESTERASE ACTIVITY

Table I shows that treatment of serum with 0.03 ml. chloroform per ml. serum activated serum TAMe esterase activity as well as a chloroform concentration twenty times as great. This holds even though the precipitate, from which the activated supernatant was decanted, was considerably smaller with the lesser chloroform concentrations. On the other hand, a concentration of 0.01 ml. chloroform per ml. serum failed to give a visible precipitate and yielded no apparent TAMe esterase activity.

Fig. 2 depicts the effect of standing at 4° on the esterase activity of chloroform treated serum as compared to the spontaneous activity of untreated serum. Although the activity of the untreated serum did not change significantly on standing, the chloroform inducible activity increased almost threefold in 4 days and maintained this level of activity for 20 days. In all experiments the time of maximum activation of the chloroform inducible esterase activity was obtained between the fourth and the seventh day. The persistence of activity was tested in two sera held at 4° for 6 weeks and 3 months respectively and no diminution in the chloroform inducible TAMe esterase activity was apparent. As shown in Fig. 3 the pH activity optimum of the chloroform inducible TAMe esterase activity after time activation was about 8.3; this is similar to that of plasmin or chloroform activation without standing (Fig. 1). It is noteworthy that the TAMe esterase activity of the chloroform preparation held for 4 days at 4° (Fig. 2) was in the same range as the plasmin activity resulting from SK activation of the same serum (Fig. 1). Furthermore, the addition of SK to serum maximally preactivated by chloroform plus standing resulted in the usual plasmin increment. Incubation of chloroform treated serum at 22° resulted in a

maximum increase that was slightly less than twofold as compared to a threefold rise in the same preparation held at 4°; incubation at 37° produced no apparent increase with time, even when activity was checked at 15 and 30 minutes.

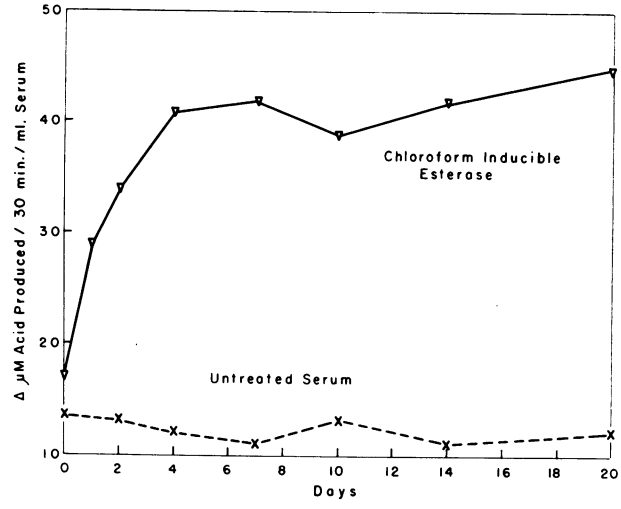


FIG. 2. *Effect of time on the chloroform inducible TAMe esterase activity of human serum.* Control serum and chloroform activated serum were placed at 4°, and the difference in their activity with time is plotted as the chloroform inducible esterase.

TABLE 2

THE EFFECT OF HEATING SERUM BEFORE ACTIVATION OF HUMAN TAMe ESTERASE ACTIVITY

| *Method of activation* | μM *acid produced per* 30 *minutes per ml. serum* | | | | *Per cent reduction in activation* |
|---|---|---|---|---|---|
| | *Unheated serum* | | *Heated serum** | | |
| | *Total activity* | *Increment due to activation†* | *Total activity* | *Increment due to activation†* | |
| None | 11 | | 4 | | |
| Chloroform | 22 | 11 | 4 | 0 | 100 |
| SK | 54 | 43 | 34 | 30 | 30 |
| Chloroform-SK | 69 | 47 | 33 | 29 | 38 |

* Serum heated at 56° for 30 minutes before activation.

† Net activity due to the last step in the activation procedure; e.g. chloroform refers to the increment inactivity obtained by chloroform treatment of plain serum, SK refers to the increment obtained by the addition of SK to plain serum, and chloroform-SK refers to the increment obtained by the addition of SK to serum pretreated with chloroform.

## COMPARISON OF THE HEAT AND ACID STABILITY OF THE CHLOROFORM AND SK ACTIVATED PROESTERASES

In Table 2 are shown the results of heating serum at 56° for 30 minutes before chloroform activation. Heating destroyed all the chloroform inducible TAMe esterase activity; even after standing for 7 days at 4° there was no apparent chloroform inducible activity. In contrast, heating serum in the same fashion before SK activation resulted in only a 30 per

cent loss in activity, and heating serum before chloroform treatment and SK activation still resulted in only a 38 per cent reduction in plasmin.

In addition, as shown in Table 3, when serum was held at pH 2.3 for 1 hour and then neutralized, there was a 74 per cent loss of the chloroform inducible TAMe esterase

TABLE 3
THE EFFECT OF ACID TREATING SERUM BEFORE ACTIVATION OF HUMAN TAMe ESTERASE ACTIVITY

| *Experiment number* | *Method of activation* | *μM acid produced per 30 minutes per ml. serum* | | | | *Per cent reduction in activation* |
|---|---|---|---|---|---|---|
| | | *Untreated serum* | | *Acid treated serum** | | |
| | | *Total activity* | *Increment due to activation†* | *Total activity* | *Increment due to activation†* | |
| 1 | None | 8 | | 13 | | |
| | Chloroform | 21 | 13 | 16 | 3 | 77 |
| | Chloroform plus 7 days at 4° | 38 | 23 | 27 | 6 | 74 |
| 2 | None | 13 | | 14 | | |
| | SK | 57 | 44 | 64 | 50 | 0 |

* Serum held at pH 2.3 and 4° for 1 hour and then neutralized.

† Net activity due to activation procedure (see footnote, Table 2). Chloroform *plus* 7 days at 4° refers to total increment obtained by chloroform treating serum and then permitting it to stand at 4° for 7 days; total corrected for activity of non-chloroform treated serum also standing 7 days. The increase in the activity of the controls on standing observed in this experiment was a rare occurrence (see Fig. 2).

TABLE 4
THE EFFECT OF SOYBEAN TRYPSIN INHIBITOR ON SERUM AND EUGLOBULIN TAMe ESTERASE ACTIVITY

| *Experiment number* | *Method of activation* | *Increment in μM acid produced per 30 minutes per ml. serum or equivalent of euglobulin due to activation** | | *Per cent inhibition* |
|---|---|---|---|---|
| | | *No SBI* | *SBI added†* | |
| 1. Serum | Chloroform | 13 | 16 | 0 |
| | SK | 33 | 15 | 55 |
| | Chloroform-SK | 47 | 16 | 66 |
| 2. Euglobulin | Chloroform‡ | 22 | 19 | 14 |
| | SK | 53 | 16 | 70 |
| | Chloroform-SK‡ | 60 | 17 | 72 |

* Net activity due to last step in activation procedure (see footnote, Table 2).

† SBI added after completion of activation process (500 μg. SBI per ml. serum or equivalent of euglobulin).

‡ Chloroform activated serum is converted to euglobulin and assayed as such or further activated with SK; this is necessary because chloroform treatment of euglobulin itself does not activate (see text).

activity but no reduction in plasmin. Thus, the precursor of the chloroform inducible esterase activity is heat and acid labile whereas plasminogen is predominantly heat and acid stable.

## COMPARISON OF THE EFFECT OF SBI ON THE CHLOROFORM AND SK INDUCIBLE TAMe ESTERASE ACTIVITY

Table 4, experiment 1, compares the effect of SBI (500 μg. per ml. serum) on the chloroform inducible esterase activity, plasmin and the plasmin of serum pretreated with

chloroform. Whereas SBI produced no inhibition of the chloroform inducible serum TAMe esterase activity, there was a 55 per cent inhibition of the plasmin of untreated serum and a 66 per cent inhibition of the plasmin of serum pretreated with chloroform. The latter indicates that any residual chloroform did not interfere with the inhibitory action of SBI.

TABLE 5
THE EFFECT OF SUBSTRATE ON ESTERASE ACTIVITY OF HUMAN SERUM

| *Method of activation* | μM *acid produced per 30 minutes per ml. serum* | | | *Ratio of TAMe: BAMe: ATEe** |
|---|---|---|---|---|
| | *TAMe* | *BAMe* | *ATEe* | |
| None | 8 | 13 | 0 | 1:1.63:0 |
| SK† | 34 | 34 | 0 | 1:1:0 |
| Chloroform† | 19 | 15 | 7 | 1:0.79:0.37 |

* Activity against TAMe arbitrarily assigned value of 1; final concentration of each substrate is 0.02 M.

† Net activity due to last step in activation procedure (see footnote, Table 2).

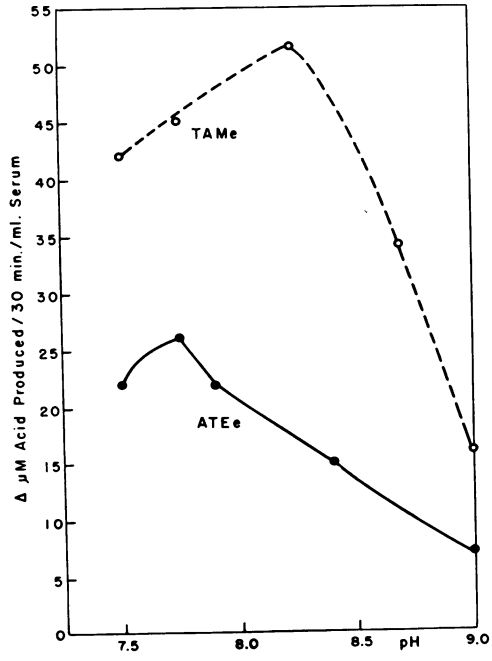


FIG. 3. *Effect of pH on the chloroform inducible TAMe and ATEe esterase activity of human serum.* The activity plotted is the chloroform induced increment when the final pH of the assay was varied as noted on the abscissa.

The second experiment, performed with serum from a different donor, compares the effect of SBI on euglobulin TAMe esterase rather than serum TAMe esterase activity. Chloroform, in contrast to SK, was not capable of activating euglobulin directly by a procedure identical with that used herein for whole serum. Hence, it was necessary to

chloroform activate serum and then convert it to euglobulin in order to obtain a preparation that could be compared with plasmin from the SK activation of euglobulin. Within these limitations, this experiment demonstrated that SBI produces only slight inhibition of the euglobulin TAMe esterase activity derived from chloroform treated serum, but

TABLE 6

THE EFFECT OF TIME ON THE SUBSTRATE SPECIFICITY OF THE CHLOROFORM INDUCIBLE ESTERASE ACTIVITY

| *Method of activation* | *Increment in μM acid produced per 30 minutes per ml. serum due to activation** | | | *Ratio of TAMe: BAMe: ATEe†* |
|---|---|---|---|---|
| | *TAMe* | *BAMe* | *ATEe* | |
| Chloroform | 19 | 15 | 7 | 1:0.79:0.37 |
| Chloroform plus 14 days at 4° | 42 | 35 | 15 | 1:0.83:0.36 |
| Chloroform plus 28 days at 4° | 44 | 35 | 18 | 1:0.80:0.41 |

* Net activity due to activation procedure (see footnotes, Tables 2 and 3).

† Activity against TAMe arbitrarily assigned a value of 1; final concentration of each substrate is 0.02 M.

TABLE 7

THE CASEINOLYTIC ACTIVITY OF CHLOROFORM AND SK ACTIVATED HUMAN SERUM

| *Experiment number* | *Method o activation* | *Increment in optical density at 280 mμ per 60 minutes per 0.5 ml. serum due to activation** | | |
|---|---|---|---|---|
| | | *No inhibitor* | *Inhibitor added SBI†* | *TAMe†* |
| 1 | Chloroform plus 10 days at 4° C.‡ | 0.005 | | |
| | SK | 0.212 | 0.018 | 0.002 |
| 2 | SK | 0.293 | 0.040 | |
| | Chloroform plus 10 days at 4° −SK§ | 0.408 | 0.025 | |

* Net activity due to last step in activation procedure (see footnote, Table 2). Plain serum had no measurable activity on casein. SK in these experiments refers to the increment in activity obtained by the addition of SK to plain serum which like the chloroform preparation had been standing at 4° for 10 days. Chloroform plus 10 days at 4° −SK refers to the increment in activity obtained by the addition of SK to serum previously activated by chloroform treatment and standing.

† SBI (500 μg. per ml. serum) and TAMe (final concentration 0.02 M) added after completion of activation process.

‡ The chloroform induced TAMe esterase activity of this preparation was 44 μM acid produced per 30 minutes per ml. serum.

§ The chloroform treated serum alone had no activity against casein, although the TAMe esterase activity of this preparation was 26 μM acid produced per 30 minutes per ml. serum.

produces a 70 per cent inhibition of the plasmin of plain euglobulin or euglobulin derived from chloroform treated serum. Thus, plasmin prepared from either whole serum or euglobulin is mostly SBI inhibited, whereas the chloroform inducible esterase activity is predominantly SBI resistant even when the serum has been converted to euglobulin.

### SUBSTRATE SPECIFICITIES OF THE CHLOROFORM AND SK INDUCIBLE SERUM ESTERASE ACTIVITY

The substrate specificities of untreated serum, plasmin and the chloroform inducible esterase activities are compared in Table 5. Although untreated serum and plasmin were active against TAMe and BAMe, neither was capable of splitting ATEe. In contrast, the chloroform activated serum was capable of splitting ATEe, as well as TAMe and BAMe. None of the three preparations exhibited significant activity against L-leucine ethyl ester, L-phenylalanine ethyl ester, or L-serine ethyl ester.

As shown in Fig. 3, the pH optimum of the maximum chloroform inducible esterase activity was about 7.8 against ATEe and 8.3 against TAMe. The chloroform inducible ATEe esterase activity like that against TAMe arose from a heat and acid labile precursor and was completely resistant to inhibition by SBI. Furthermore, the data in Table 6 show that the relative activities of the chloroform activated serum against TAMe, BAMe and ATEe did not change with standing at 4° for 2 or 4 weeks even though there was a greater than twofold increase in esterase activity.

### CASEINOLYTIC ACTIVITY OF CHLOROFORM AND SK ACTIVATED SERUM

The data in Table 7 reveal that, despite maximum TAMe esterase activation by chloroform treatment and standing at 4°, serum prepared by this technique was unable to split casein. In contrast, SK activated serum was caseinolytic even though the TAMe esterase activity was no greater than that of the chloroform preparation which failed to split casein. Furthermore, while chloroform treated serum was not caseinolytic, the addition of SK to such serum produced caseinolytic activity that exceeded that obtained by the similar addition of SK to plain serum.

### THE EFFECT OF IMMUNE PRECIPITATES ON THE CHLOROFORM AND SK INDUCIBLE ESTERASE ACTIVITY OF HUMAN SERUM

Lepow, Ratnoff and Pillemer (1956) demonstrated that an esterase could be eluted from immune precipitates exposed to human serum; this eluate arose from a heat labile preesterase, split TAMe and ATEe but not casein and was not inhibited by SBI. The similarity between this eluate and the chloroform inducible esterase activity suggested that pretreatment of serum with an antigen-antibody precipitate might diminish the esterase activity resulting from subsequent chloroform activation. That this was the case is illustrated in Table 8. Pretreatment of serum with an immune precipitate reduced the maximal chloroform inducible TAMe esterase activity from 28 to 19 μM and the maximal inducible ATEe esterase activity from 16 to 4 μM of acid produced per 30 minutes per ml. serum. In contrast, pretreatment of serum with the immune precipitate did not diminish the SK inducible TAMe esterase activity of plain serum or serum pretreated with chloroform.

As shown in Table 8, the μM of chloroform inducible esterase activity missing per 30 minutes per ml. serum due to exposure to the immune precipitate was 9 of TAMe and 12 of ATEe esterase respectively. In Table 9 this loss is expressed per 5.0 ml. serum so that it can be compared with the esterase activity taken up by the precipitate to which the serum was exposed. The data reveal that the TAMe and ATEe esterase activity of the precipitate represents only 47 and 43 per cent recovery respectively of the missing chloroform inducible esterase activity. None the less, as might be expected, the ratio of TAMe to ATEe esterase activity missing from serum, 1/1.33, as estimated by maximal

chloroform activation, was very similar to the ratio of TAMe to ATEe esterase activity taken up by the precipitate, 1/1.24. Although the esterase activity induced by chloroform

TABLE 8

EFFECT OF PRETREATING HUMAN SERUM WITH AN ANTIGEN-ANTIBODY PRECIPITATE ON CHLOROFORM INDUCIBLE TAMe AND ATEe ESTERASE ACTIVITY

| *Experiment number* | *Method of activation* | *Increment in μM acid produced per 30 minutes per ml. serum due to activation** | | *μM esterase activity removed per ml. serum* |
|---|---|---|---|---|
| | | *Untreated serum* | *Serum treated with immune precipitate†* | |
| 1. TAMe | Chloroform | 11 | 5 | 6 |
| | Chloroform plus 7 days at 4° | 28 | 19 | 9 |
| | SK | 28 | 29 | 0 |
| | Chloroform-SK | 42 | 43 | 0 |
| 2. ATEe | Chloroform | 4 | 1 | 3 |
| | Chloroform plus 7 days at 4° | 16 | 4 | 12 |

* Net activity due to last step in activation procedure (see footnote, Tables 2 and 3).

† 0.30 mg. of rabbit antibovine serum albumin antibody nitrogen per ml. human serum.

TABLE 9

THE UPTAKE OF TAMe AND ATEe ESTERASE ACTIVITY BY BOVINE SERUM ALBUMIN RABBIT ANTIBOVINE SERUM ALBUMIN PRECIPITATE TREATED WITH HUMAN SERUM*

| | *Substrate* | |
|---|---|---|
| | *TAMe* | *ATEe* |
| μM acid produced per 30 minutes by precipitate exposed to 5 ml. serum | 21 | 26 |
| μM of chloroform inducible esterase activity removed per 5 ml. serum by precipitate as estimated by chloroform activation plus standing 7 days at 4°† | 45 | 60 |
| Per cent of missing chloroform inducible esterase activity taken up by precipitate | 47 | 43 |
| Ratio of serum chloroform inducible TAMe : ATEe esterase activity‡ | 1/0.57 | |
| Ratio of serum chloroform inducible TAMe : ATEe esterase activity missing from serum due to exposure to the precipitate | 1/1.33 | |
| Ratio of TAMe : ATEe esterase activity taken up by the precipitate | 1/1.24 | |

* Same experiment as described in Table 8.

† μM esterase activity removed per ml. serum is recorded in Table 8; this was multiplied by five for use in Table 9.

‡ Activity against TAMe arbitrarily assigned value of 1.

and that of the immune precipitate exhibited a different ratio of activity against TAMe and ATEe (Table 9), the pH optimum for both was 8.3 against TAMe and 7.8 against

ATEe* (Figs. 3 and 4). Furthermore, the esterase activity of the immune precipitate, like that induced by chloroform, was not inhibited by SBI and did not appear if the serum to which the precipitate was exposed had been previously heated at 56° C. for 30 minutes.

The difference in the activity ratio† of the chloroform and the antigen-antibody induced esterase activity suggested that more than one esterase might be activated by one or both procedures. Hence, the same serum sample was treated serially with three fresh immune

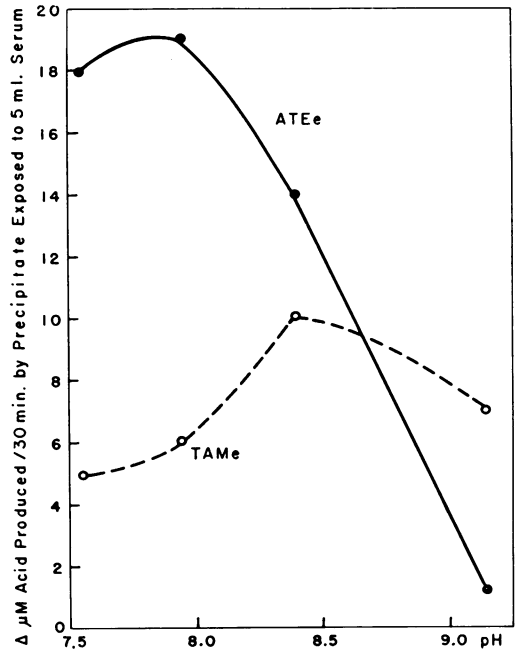


FIG. 4. *Effect of pH on the TAMe and ATEe esterase activity of the antigen-antibody precipitate.* The activity taken up by the immune precipitate, 0.15 mg. rabbit anti-bovine serum albumin nitrogen per ml. human serum (see text), was estimated at a variety of buffer pHs; the final pH of the assay mixture was as recorded on the abscissa.

precipitates to see if the ratio of TAMe to ATEe taken up by the precipitates changed or if one activity could be completely removed from serum leaving primarily the other. The data presented in Table 10 show that the ratio of TAMe to ATEe esterase activity taken up by the three precipitates was virtually identical. As illustrated in Table 11, the serial treatment removed 48 and 98 per cent respectively of the chloroform inducible TAMe and ATEe esterase activity, while the total activity picked up by the three precipitates

* The chloroform, peptone and antigen-antibody induced ATEe esterase activity as recorded in all tables is based on a measurement at pH 8.3 rather than at the pH optimum of 7.8; this was done so that the ratio of TAMe : ATEe esterase activity at one pH could be presented. Measurement of the ATEe esterase activity at pH 7.8 in each of these circumstances does not alter the percentage of inducible activity missing from serum due to exposure to the precipitate, the percentage of missing activity taken up by the precipitate, or the fact that the chloroform inducible activity ratio against TAMe : ATEe is different from that resulting from peptone or antigen-antibody activation.

† The ratio of the chloroform inducible serum TAMe : ATEe esterase activity was found to range from 1 : 0.37 to 1 : 0.69, whereas the ratio of the antigen-antibody inducible activity ranged from 1 : 1.24 to 1 : 1.42. The variation in both instances can probably be attributed to variation within the donor group and to small changes in the pH of the buffer.

accounted for 65 and 62 per cent respectively of the missing chloroform inducible TAMe and ATEe esterase activity. The ratio of TAMe to ATEe esterase activity missing from serum, 1/1.42, and that found on the three precipitates, 1/1.43, were again similar,

TABLE 10
THE UPTAKE OF TAMe AND ATEe ESTERASE ACTIVITY BY BOVINE SERUM ALBUMIN RABBIT ANTIBOVINE SERUM ALBUMIN PRECIPITATES WHEN THE SAME SERUM SAMPLE IS TREATED SERIALLY WITH THREE FRESH PRECIPITATES

| *Precipitates** | *μM acid produced per 30 minutes by precipitate exposed to 5 ml. serum* | | *Ratio of TAMe:ATEe†* |
|---|---|---|---|
| | *TAMe* | *ATEe* | |
| Number 1 | 19 | 27 | 1:1.42 |
| Number 2 | 16 | 23 | 1:1.44 |
| Number 3 | 7 | 10 | 1:1.43 |

* 0.15 mg. of rabbit antibovine serum albumin antibody nitrogen per ml. human serum.
† Activity against TAMe arbitrarily assigned value of 1.

TABLE 11
THE UPTAKE OF TAMe AND ATEe ESTERASE ACTIVITY BY BOVINE SERUM ALBUMIN RABBIT ANTIBOVINE SERUM ALBUMIN PRECIPITATES WHEN THE SAME SERUM SAMPLE IS TREATED SERIALLY WITH THREE FRESH PRECIPITATES*

| | *Substrate* | |
|---|---|---|
| | *TAMe* | *ATEe* |
| μM acid produced per 30 minutes by sum of the three precipitates exposed to 5 ml. serum (Table 10) | 42 | 60 |
| Increment in μM acid produced per 30 minutes per 5 ml. serum due to chloroform treatment and standing 7 days at 4° | 150 | 104 |
| Increment in μM acid produced per 30 minutes per 5 ml. serum exposed to the three precipitates prior to chloroform treatment and standing 7 days at 4° | 78 | 2 |
| μM of chloroform inducible esterase activity missing due to exposure to the three precipitates | 72 | 102 |
| Per cent of chloroform inducible esterase activity missing due to exposure to the three precipitates | 48 | 98 |
| Per cent of missing chloroform inducible esterase activity taken up by the three precipitates | 65 | 62 |
| Ratio of serum chloroform inducible TAMe : ATEe esterase activity† | 1:0.69 | |
| Ratio of serum chloroform inducible TAME : ATEe esterase activity missing from serum due to exposure to the three precipitates | 1:1.42 | |
| Ratio of TAMe : ATEe esterase activity taken up by precipitates | 1:1.43 | |

* Same experiment as described in Table 10.
† Activity against TAMe arbitrarily assigned value of 1.

whereas the chloroform inducible esterase activity against TAMe and ATEe gave a ratio of 1/0.69. In another experiment in which serum was exposed five times to fresh immune precipitates, the activity ratio was again virtually identical in the first three precipitates.

However, the activity of the last two was negligible, suggesting that either the ATEe and TAMe esterase activity of the precipitates arose from the same precursor or that the relative affinity of the two preesterases for the precipitates did not change with serial exposure. Furthermore, the fact that three serial treatments again removed essentially all the chloroform inducible ATEe esterase and about 50 per cent of the chloroform inducible TAMe esterase activity, while the fourth and fifth precipitates failed to pick up any of the residual TAMe esterase activity, suggests that this residual chloroform inducible TAMe esterase activity differs from that taken up by the precipitates.

TABLE 12

EFFECT OF SUBSTRATE ON CHLOROFORM INDUCIBLE ESTERASE ACTIVITY AFTER CONVERSION OF CHLOROFORM TREATED SERUM TO EUGLOBULIN AND STANDING AT 4°

| *Time of standing after conversion* | *Increment in μM of acid produced per 30 minutes per ml. of 1:1 euglobulin due to activation** | | *Ratio of TAMe:ATEe†* |
|---|---|---|---|
| | *TAMe* | *ATEe* | |
| No standing | 14 | 15 | 1:1.07 |
| 48 hours at 4° | 28 | 18 | 1:0.64 |
| 7 days at 4° | 43 | 14 | 1:0.33 |

* Net increase in euglobulin activity due to chloroform treatment of serum, conversion to euglobulin, and standing at 4°; total corrected for activity of euglobulin prepared from plain serum and placed at 4° for the same period of time as the chloroform preparation.

† Activity against TAMe arbitrarily assigned value of 1.

TABLE 13

THE EFFECT OF CONVERSION TO EUGLOBULIN ON THE CHLOROFORM INDUCIBLE ATEe ESTERASE ACTIVITY OF SERUM

| *Method of activation* | *Increment in μM acid produced per 30 minutes per ml. serum or equivalent of euglobulin** | | |
|---|---|---|---|
| | *No standing* | *4 days at 4°* | *7 days at 4°* |
| Chloroform-serum | 4 | 13 | 24 |
| Chloroform-euglobulin† | 13 | 14 | 14 |

* Net increase due to chloroform activation (see footnote, Table 2).

† Chloroform activated serum is converted to euglobulin and assayed as such or after standing for a given period of time at 4° (see footnote, Table 12).

## THE EFFECT OF TIME ON THE FURTHER ACTIVATION OF EUGLOBULIN PREPARED FROM CHLOROFORM TREATED SERUM

The effect of standing at 4° on the TAMe and ATEe esterase activity of euglobulin prepared from chloroform activated serum is presented in Table 12. The TAMe esterase activity of the euglobulin increased threefold on standing but there was no apparent increment in the ATEe esterase activity. The latter is in marked contrast to the finding in whole serum wherein the relative increase in TAMe and ATEe esterase activity is identical with time (Table 6). Furthermore, whereas the TAMe esterase activity of the euglobulin before standing was approximately equal to that of the chloroform activated serum from

which it was derived, the ATEe esterase activity of the euglobulin before standing was much greater than that of the serum from which it was prepared (Table 13). Thus, the conversion of chloroform activated serum to euglobulin leaves the precursor of the chloroform inducible TAMe esterase activity intact allowing the further activation characteristic of whole serum to proceed. On the other hand, the same procedure apparently activates the precursor of the chloroform inducible ATEe esterase, possibly by removing an inhibitor, leaving no apparent precursor for further activation with time.

Studies with resin treated serum also demonstrated a partial dissociation between the chloroform inducible TAMe and ATEe esterase activities. Resin treatment before activation did not diminish the chloroform inducible ATEe esterase activity, but greatly reduced the chloroform inducible TAMe esterase activity; the latter was not restored by recalcification. In a typical experiment the chloroform inducible ATEe esterase activity of control serum, resin treated serum and recalcified ($2.5 \times 10^{-3}$ M) resin treated serum was virtually identical after standing at 4° for 7 days—21 μM acid produced per 30 minutes per ml. serum. In contrast, the chloroform inducible TAMe esterase activity of the control serum, 46 μM acid produced per 30 minutes per ml. serum, greatly exceeded that of the resin treated serum, which was 17 μM acid produced per 30 minutes per ml. serum even after recalcification. Since resin treatment removed more than half the chloroform inducible TAMe esterase activity, the residual chloroform inducible esterase activity, like that taken up by the immune precipitate, was more active against ATEe than against TAMe. Furthermore, it was observed that the ATEe and TAMe esterase activity taken up by the immune precipitate was the same in control serum, resin treated serum, and recalcified resin treated serum.

Thus, the conversion of chloroform activated serum to euglobulin leaves only the precursor of the chloroform inducible TAMe esterase activity intact, as measured by further activation with time, while resin treatment of serum before activation leaves only the precursor of the chloroform inducible ATEe esterase activity completely intact. These studies, and those with the immune precipitate, suggest that the chloroform inducible esterase activity is made up of at least two components; one is predominantly active against TAMe and is probably not affected by the immune precipitate, whereas the other is more active against ATEe than against TAMe and is removed from serum by exposure to the immune precipitate.

### THE EFFECT OF IMMUNE PRECIPITATE ON THE PEPTONE INDUCIBLE ESTERASE ACTIVITY OF HUMAN SERUM

Although the peptone induced esterase activity of whole serum was variable and small, the increment obtained after conversion to euglobulin, which probably removed an inhibitor present in peptone (Astrup and Olesen, 1957), was consistent and sufficient for study. No further increment in esterase activity was obtained by allowing the peptone euglobulin preparation to stand at 4° for 48 hours or 7 days. The peptone inducible esterase, like the chloroform inducible activity, arose from a precursor which was destroyed by heating at 56° for 30 minutes or lowering the pH to 2.3 for 1 hour, was not inhibited by SBI (500 μg. per ml. serum), had a pH optimum of 8.3 for TAMe and 7.8 against ATEe, and was not capable of splitting casein, even though the addition of SK to such a preparation imparted caseinolytic activity.

There were, however, some differences between the peptone and the chloroform inducible esterase activities when both were studied in their euglobulin preparation.

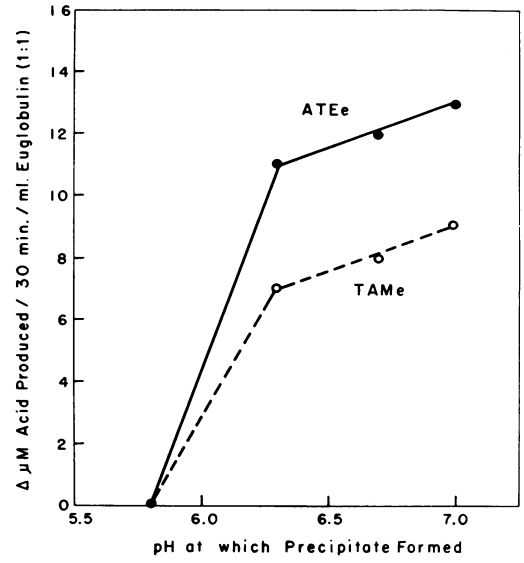


FIG. 5. *The relationship of the pH at which peptone activated serum is precipitated to the peptone induced TAMe and ATEe esterase activity obtained in the euglobulin precipitate.* The activity plotted is the peptone induced increment in euglobulin TAMe and ATEe esterase activity when peptone treated serum is converted to euglobulin at the pHs noted on the abscissa.

TABLE 14

EFFECT OF PRETREATING HUMAN SERUM WITH A BOVINE SERUM ALBUMIN RABBIT ANTIBOVINE SERUM ALBUMIN PRECIPITATE ON THE PEPTONE INDUCIBLE TAMe AND ATEe ESTERASE ACTIVITY OF EUGLOBULIN OBTAINED FROM THE PEPTONE TREATED SERUM*

| | *Substrate* | |
|---|---|---|
| | *TAMe* | *ATEe* |
| μM acid produced per 30 minutes by precipitate exposed to 5 ml. serum† | 22 | 30 |
| Increment in μM acid produced per 30 minutes per 5 ml. euglobulin due to peptone activation | 61 | 95 |
| Increment in μM acid produced per 30 minutes per 5 ml. euglobulin when serum exposed to precipitate prior to peptone activation | 32 | 50 |
| μM of peptone inducible esterase activity missing due to exposure to the precipitate | 29 | 45 |
| Per cent of peptone inducible esterase activity missing due to exposure to the precipitate | 48 | 47 |
| Per cent of missing peptone inducible esterase activity taken up by precipitate | 76 | 67 |
| Ratio of peptone inducible TAMe : ATEe esterase activity measured in euglobulin‡ | 1 : 1.56 | |
| Ratio of peptone inducible TAMe : ATEe esterase activity missing from euglobulin due to exposure of serum to precipitate | 1 : 1.55 | |
| Ratio of TAMe : ATEe esterase activity taken up by precipitate | 1 : 1.36 | |

* Peptone activated serum is converted to euglobulin (1 : 1) before determination of the peptone induced increment in activity.

† 0.15 mg. of rabbit anti-bovine serum albumin antibody nitrogen per ml. human serum.

‡ Activity against TAMe arbitrarily assigned value of 1.

Whereas chloroform activated serum could be converted to euglobulin by dilution and acidification to pH 5.2 without loss of TAMe esterase activity, similar treatment of peptone activated serum completely destroyed or inhibited the peptone inducible esterase activity (Fig. 5). Furthermore, the chloroform euglobulin preparation initially had approximately equal activity against TAMe and ATEe and evolved considerable TAMe esterase activity on standing at 4°, while the peptone euglobulin preparation was considerably more active against ATEe than against TAMe and demonstrated no further increase in esterase activity on standing at 4°. The latter difference may be due to the fact that chloroform activates a second TAMe esterase.

Despite these differences, the effect of pretreating serum with an immune precipitate on subsequent peptone activation was very similar to the effect of pretreating serum with an immune precipitate before chloroform activation. As illustrated in Table 14, the ratio of peptone inducible TAMe to ATEe esterase activity missing from serum after exposure to the immune precipitate, 1/1.55, and that taken up by the precipitate, 1/1.36, was very similar and like that observed in the chloroform studies (Tables 9 and 11). Furthermore, the ratio of the peptone inducible TAMe to ATEe esterase activity, 1/1.56, was similar to that taken up by the immune precipitate.

## DISCUSSION

Although both the chloroform inducible esterase activity and the plasmin activity of human serum have a similar pH optimum on TAMe, they differ in, at least, six other respects. (1) The preenzyme of the chloroform inducible esterase activity is destroyed by heating serum at 56° for 30 minutes, whereas plasminogen is predominantly stable to such treatment (Table 2). (2) The preenzyme of the chloroform inducible esterase activity is mostly destroyed by acid treatment whereas plasminogen is stable (Table 3). (3) The chloroform inducible esterase activity is completely resistant to SBI whereas plasmin is mostly inhibited (Table 4). (4) Although these preparations differ somewhat in the ratio of their activities against TAMe and BAMe, the really striking difference is that chloroform inducible esterase activity is present against ATEe while plasmin is inactive on this substrate (Table 5). (5) Plasmin exhibits caseinolytic activity but chloroform activated serum does not, even when the TAMe esterase activity of the two preparations is identical (Table7). (6) Pretreatment of serum with an antigen-antibody precipitate greatly diminishes esterase activity resulting from chloroform activation, but does not diminish that from SK activation (Table 8). In addition, the observation that the SK induced increment in TAMe esterase activity is the same in plain serum as in serum maximally activated by chloroform plus standing suggests that the esterase activities arise from different preenzymes.

Although the method of chloroform activation used here failed to induce caseinolytic activity in serum, Ratnoff (1948, a and b) using a different experimental procedure has demonstrated that such activation is obtainable. This discrepancy may be a consequence of the different activating techniques or the result of a difference in the starting materials. In the procedure used by Ratnoff (1948, a and b) euglobulin prepared from plasma is exposed to chloroform for 16 to 28 hours at 25°, in order to allow clot formation and lysis, before centrifuging to obtain the active protease in the supernatant. In contrast, during the procedure used in the present work serum is exposed to chloroform for only 4 minutes before separation by centrifugation is begun. Furthermore, the fact that fibrin strongly

absorbs and protects plasmin from inhibitors (Astrup, 1956; Mullertz, 1953) may be of importance, since clot formation and dissolution occurs during activation by the Ratnoff technique but not during the procedure described herein. On the other hand, although the chloroform preparation used in the present work is not caseinolytic, the addition of SK to such a preparation induces more caseinolytic activity than the addition of SK to plain serum.

While the chloroform inducible esterase activity is different from that of plasmin, it bears a similarity to activated $C'_1$, as characterized by Lepow, Ratnoff and Pillemer (1956). These workers demonstrated that an esterase can be eluted from immune precipitates pretreated with human serum and presented evidence that the eluate is capable of splitting TAMe and ATEe but not casein, destroys $C'_2$ and $C'_4$, is not inhibited by SBI and arises from a heat labile precursor. Earlier work by Lepow, Ratnoff, Rosen and Pillemer (1956) had revealed that a preparation of partially purified $C'_1$, under certain conditions of pH and ionic strength, loses its hemolytic potential while acquiring esterase and anticomplementary activity. Since the anticomplementary and esterase activity of the partially purified $C'_1$ could not be distinguished from that of the eluate, these authors concluded that the eluate factor probably represented activated $C'_1$. Becker (1956, a and b) studying guinea-pig serum by a different approach has also concluded that $C'_1$ can exhibit esterase activity. The chloroform inducible esterase activity, like activated $C'_1$, arises from a heat labile precursor, splits TAMe and ATEe, is not inhibited by SBI, and, most important of all, is partially removed by exposing serum to an immune precipitate before chloroform activation. Furthermore, it has been demonstrated that chloroform activated serum is anticomplementary in that it destroys $C'_2$ and $C'_4$ (Pillemer *et al.*, 1953; Lepow, Pillemer and Ratnoff, 1953).

Although the similarity between $C'_1$ and the chloroform inducible esterase activity suggests that chloroform may activate $C'_1$, the difference in their relative activities against TAMe and ATEe is somewhat against such a formulation. Whereas activated $C'_1$ (Ratnoff and Lepow, 1957) and the enzyme induced by an immune precipitate are more active against ATEe than against TAMe, the reverse is true for the chloroform inducible activity. However, the evidence presented that chloroform induces two esterase activities may resolve this discrepancy; one is predominantly a TAMe esterase and is minimally or not at all altered by exposing serum to an immune precipitate before chloroform activation, whereas the other is more active against ATEe than against TAMe, is removed by exposing serum serially to immune precipitates before chloroform activation, and resembles activated $C'_1$ (Ratnoff and Lepow, 1957) in many respects.

Peptone, unlike chloroform, activates ATEe and TAMe esterase activity in the same ratio as the immune precipitate. Furthermore, the peptone inducible esterase like that induced by chloroform and by the immune precipitate arises from a heat labile precursor, has a pH optimum of 8.3 against TAMe and 7.8 against ATEe, is not inhibited by SBI, and does not digest casein.

The finding that the immune precipitate takes up esterase activity as well from resin treated as from control serum is consistent with the observation of others (Lepow *et al.*, 1954) that an immune precipitate,* 0.20 mg. antibody nitrogen per ml. human serum, can inactivate complement as readily in resin treated as in control human serum. Both the chloroform and the peptone inducible esterase activity of resin treated serum, like that taken up by the immune precipitate, were more active against ATEe than against TAMe.

* Pneumococcal type III rabbit antipneumococcal type III precipitate.

Whether these esterase activities arise from the same or merely a similar preesterase is not established; however, the fact that pretreatment of serum with an immune precipitate removes significant quantities of the chloroform and peptone inducible activity makes it likely that the same preesterase is involved. Although the chloroform, peptone and antigen-antibody induced esterase activity differs from plasmin and resembles activated $C'_1$, there is no conclusive evidence that this activity really is activated $C'_1$.

The failure of the immune precipitate to activate human plasminogen does not invalidate the concept that the antigen-antibody reaction induces other enzymic activity which plays a role in anaphylaxis. The finding with respect to plasminogen in man duplicates earlier work with guinea-pig serum (Austen, Becker and Marcus, 1959; Becker, Austen and Marcus, 1959), and is merely additional evidence that it is not the plasmin system which is responsible. The evidence that plasmin does not play a fundamental role in anaphylaxis has recently been reviewed by Burdon (1958) and by Becker (1957).

The studies of Humphrey and Jaques (1955) are particularly pertinent to the esterase work because these workers demonstrated that chloroform, peptone or antigen-antibody activated rabbit serum released histamine from rabbit platelets while the addition of SK or SK activated human serum failed to release histamine. The addition of specific antigen to rabbit platelets suspended with purified antibody resulted in histamine release only if intact serum was present; antigen failed to release histamine if the serum had been resin treated or heated to 56° for 30 minutes. The antigen induced histamine release was not associated with the appearance of demonstrable thrombolytic activity, whereas SK activated rabbit or human serum was thrombolytic but failed to release histamine. Chloroform or peptone activated rabbit serum exhibited both proteolytic and histamine releasing capacity. The inability of plasmin to release histamine, and the absence of demonstrable thrombolytic activity after histamine release by specific antigen, suggest that either the histamine releasing ability of the chloroform or peptone activated serum does not reside in its proteolytic capacity or that the responsible protease is only transiently present. The fact that calcium is required for histamine release in rabbit serum (Humphrey and Jacques, 1955) but not for esterase activation or activity in human serum could mean that these phenomena are not related; however, there are a number of other possible explanations. The calcium requirement in the rabbit experiments could be the result of a species difference; the calcium requiring step in histamine release could be subsequent to esterase activation; the time interval between resin treatment and assay may have been prolonged in the rabbit work, for it has been demonstrated that human $C'_1$ deteriorates if resin treated serum stands in the absence of calcium (Lepow *et al.*, 1953, 1954); or the extent of decalcification with the resin may not have been the same in both experiments.*

In view of the evidence that chloroform, peptone and antigen-antibody activate similar, if not identical, preesterases, it is tempting to speculate that this enzymic activity plays a role in histamine release. The observation that peptone shock in the dog significantly resembles anaphylaxis lends some support to this suggestion.

## ACKNOWLEDGMENT

The expert technical assistance of Miss Nina Glass is gratefully acknowledged.

* Although calcium was not detectable in the reaction mixture by the EDTA method (Elliot, 1952), the possibility that a trace of calcium was present during the esterase studies with resin treated human serum cannot be ruled out.

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