THE NATURE OF SERUM ANTITRYPSIN.

STUDIES ON FERMENT ACTION. XIII.*

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The efforts that have been made to determine the nature of the ferment-inhibiting substances of the blood give no definite information concerning their significance or their character. At one time it was thought that the antitryptic index of the serum would prove to be a valuable aid in the diagnosis of cancer.

Brieger and Trebing (1) stated that 90 per cent. of the patients suffering from carcinoma or sarcoma, whom they had examined, showed an increase of antitrypsin in the blood. Von Bergmann and Meyer (2) confirmed this observation, though they also found a similar increase in 24 per cent. of non-cancerous patients. Recent work indicates that the increased ferment-inhibiting action of the serum cannot be relied upon as a diagnostic test for cancer. It is frequently present in the acute infections, such as pneumonia, typhoid fever, etc.; in chronic infections, such as tuberculosis and syphilis; in Graves' disease; and in severe anemias. The action of the serum has been ascribed to chemical constituents and to specific immune bodies which act as antiferments. Meyer (3) believes that the antiferment is a true antibody, and that the ferments of the tissue cells act as antigens. He concludes that trypsin and leucoprotease are not so important in this respect. Eisner (4) and Wiens (5) also believe that the antiferment is a true antibody, but that the antigen is the ferment liberated by the polymorphonuclear cells. On the other hand, Jürgensen (6) found no relation between the antitryptic index and leucocytosis. Halpern (7) inoculated dogs with the pancreas of dogs, and found an increase in antitrypsin, but no increase in antipepsin. Eisner (4) studied the inhibiting action of serum against various ferments, and concluded that it possessed a special affinity for trypsin. Some writers have asserted that the serum is more active against trypsin of the same species, but Weil (8) and others have disproved this.

Morgenroth (9) thought that he obtained a specific antirenin by inoculating animals with renin, while Achalme (10) states that he produced a specific antitrypsin by inoculating animals with trypsin. Other investigators repeated the work of Morgenroth and Achalme and have failed to confirm their results. Hamburger (11) showed that sodium chloride destroys pepsin in a neutral solution, thereby invalidating the evidence that a specific antipepsin is present in the blood; immune bodies are active only in a neutral or slightly alkaline reac-

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tion. We must conclude, then, that sufficient proof has not been advanced to support the view that the ferment-inhibiting substances of the blood are true antibodies.

Hedin (12) has shown that charcoal will bind trypsin and that the binding is proportional to the quantity of charcoal and to the time of interaction. Strong acids and alkalies quickly destroy trypsin, but Chittenden and Cummins (13) found it to be much more resistant to these agents in the presence of protein. Kudo (14) states that all the mineral acids are active in this respect, sulphuric acid being active in a dilution of one in a thousand, while the organic acids are much less so. Bayliss (15) and Abderhalden and Gigon (16) found that some of the products of tryptic activity possess the property of inhibiting the action of the ferment, the free amino acids being more active than the polypeptids.

Schwartz (17) concluded that the antitryptic activity of the serum is due to the lipoids. He found that he could partially inactivate sera by extracting them with ether, and that the sera could be reactivated by the addition of lecithin. Sugimoto (18) observed a decrease in the antitryptic strength of sera after extraction with ether and benzol, and concluded that the lipoids were the active constituents. He believes that a complete extraction of the fat, and the dissociation of the lipoid-protein combinations will cause a removal of the ferment-inhibiting action. He also tested alcoholic extracts which were prepared from egg-white, beef brain, and beef liver, and found them inactive as anti-enzymes when used as emulsions in solutions of sodium chloride. Meyer (3) suggests that the effect of the ether was due to the destruction of the anti-enzymes and not to their extraction. He also refers to the normal antitryptic index found in the lipoid-rich sera of luetics and diabetics. He found that it was not destroyed by extracting the dried serum with ether, and the fluid serum with petroleum ether, but states that this does not disprove the possibility of its being a lipoid-protein combination. He does not believe that protein cleavage products are the inhibiting agents. Cobliner (19) assumes that lipoids are not the active agents, as dried sera extracted with ether, chloroform, or petroleum ether do not lose their antitryptic activity. He found that the inhibiting action was increased if the serum was shaken with olive oil. Kirchheim (20) concludes that the antiferment merely prolongs the action of the trypsin and does not destroy it. He observed that chloroform reduced the inhibiting action of the serum.

Rosenthal (21) believes that the action of the serum is due to protein cleavage products, and that its increase in carcinoma and other diseases with cachexia can be thus explained.

Two years ago we observed that sera preserved with chloroform soon lost their antitryptic action. The same observation had been made previously by Delezenne and Pozerski (22), but we did not know of it. This discovery convinced us that the ferment-inhibiting substances of the serum were lipoids, and that they were soluble in fat solvents. We (23) have published the results of some preliminary work based on this idea, in which it was shown that the soaps of the unsaturated fatty acids obtained from various sources possessed the property of acting as anti-enzymes. These results caused us to study the ferment-inhibiting action of the blood to see if it was due to the same agents.

The Fuld-Gross technique was used by most of the investigators engaged in the study of the anti-enzymes of the blood. We have already discussed in

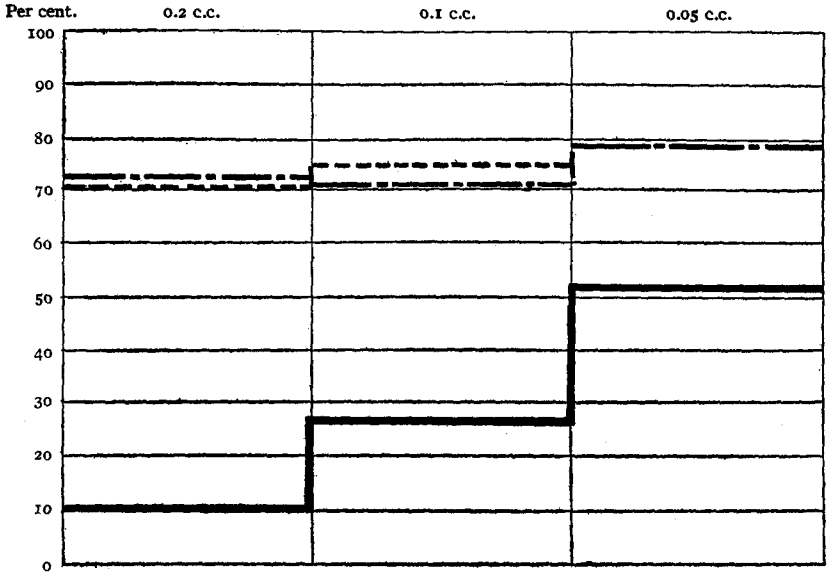
another paper (23) the difficulties we encountered in the use of this method. In this work, as well as in that previously reported, our interpretations were based upon the total incoagulable nitrogen determined according to the method recommended by Folin.

INFLUENCE OF CHLOROFORM OR ETHER EXTRACTION ON SERUM ANTITRYPSIN.

Considerable work has been done with lipoid solvents in order to show the relation which exists between the lipoids of the serum and antitrypsin, but with contradictory results. In most of the work the solvent was allowed to act for a short time only, and, in some instances, on the dried serum. In the first experiment we wished to determine the influence of chloroform or ether on the enzyme-inhibiting action of the serum. Fresh dog serum was diluted 1 to 5 with salt solution, and divided into three parts. To one part was added chloroform; to another ether; the remaining portion was preserved without the addition of any agent. The three flasks containing the sera were then kept at room temperature for forty-eight hours. The mixtures were shaken frequently during this time. Before testing, the excess of chloroform or ether was removed with pipettes, and the serum then filtered through filter paper until clear. They were mixed in various dilutions with trypsin and placed in the incubator for thirty minutes. Casein was then added and the mixture incubated for two hours at 37° C. A control flask containing only trypsin and casein was incubated for the same period of time. The lines on text-figure 1 show the amount of digestion in each flask as compared with that obtained in the control tube which contained no serum. This method will be followed in all the subsequent experiments.

Text-figure 1 shows the results of extracting the serum with ether or chloroform. The lines in the text-figure show that in the tube containing 0.2 of a cubic centimeter of the unextracted serum, the digestion was only 10 per cent. of that obtained in the control, while in the tubes containing the extracted serum, the digestion was 70 per cent. of the control. In other words, the chloroform and ether had removed 60 per cent. of the inhibiting action of the serum. Dog serum has not the high anti-enzyme power of human serum, and this accounts for the comparatively large amount

used. This experiment has been repeated many times with human serum, horse serum, dog serum, and guinea pig serum, and similar results have been obtained with each. It was found necessary to shake the mixtures of chloroform and ether frequently, otherwise the inhibiting action of the serum was not removed. At least twice as much solvent as serum should be used.



Black line = original serum.
Dotted line = ether-extracted serum.
Broken line = chloroform-extracted serum.

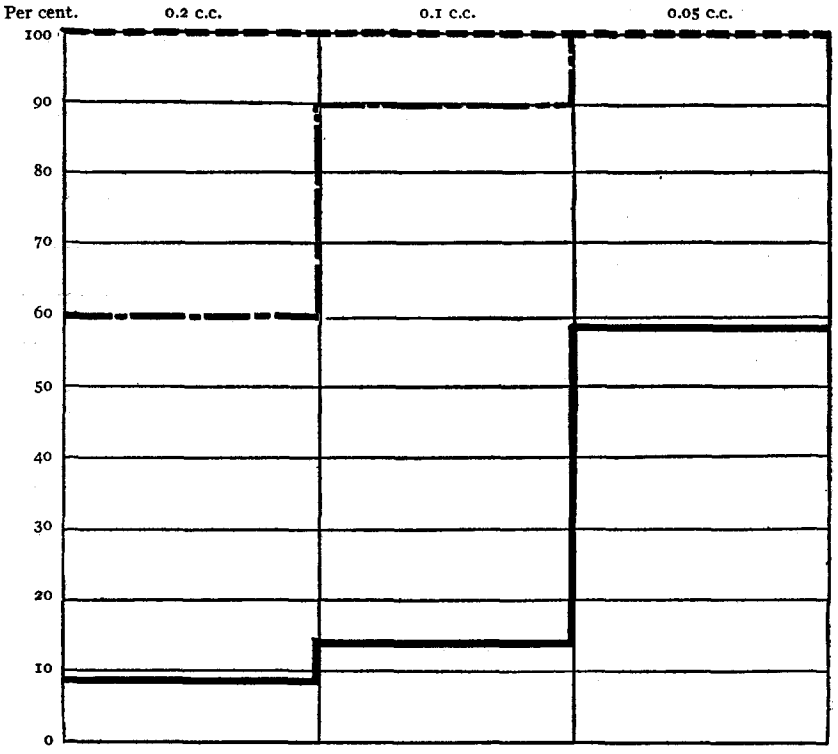
TEXT-FIG. 1. Effect of ether or chloroform extraction on serum antitrypsin.

Two possibilities must be considered in explaining the action of chloroform or ether on the enzyme-inhibiting action of the serum: first, that the substances causing the inhibiting action have been destroyed; and, second, that they have been taken up by the solvents.

INFLUENCE OF THE REACTION ON EXTRACTED SERA.

The next experiment was conducted to see if the activity of the serum could be restored by rendering it neutral or slightly alkaline in reaction. In order to duplicate the conditions present in the last experiment, ether was added to the serum in two tubes, and the

mixtures were kept at room temperature. A third tube of serum was kept without any preservative. After four days the ether was evaporated from one tube, the serum shaken thoroughly, and then made slightly alkaline with a dilute solution of sodium hydrate. This was done to see if the acids could be saponified, and the activity



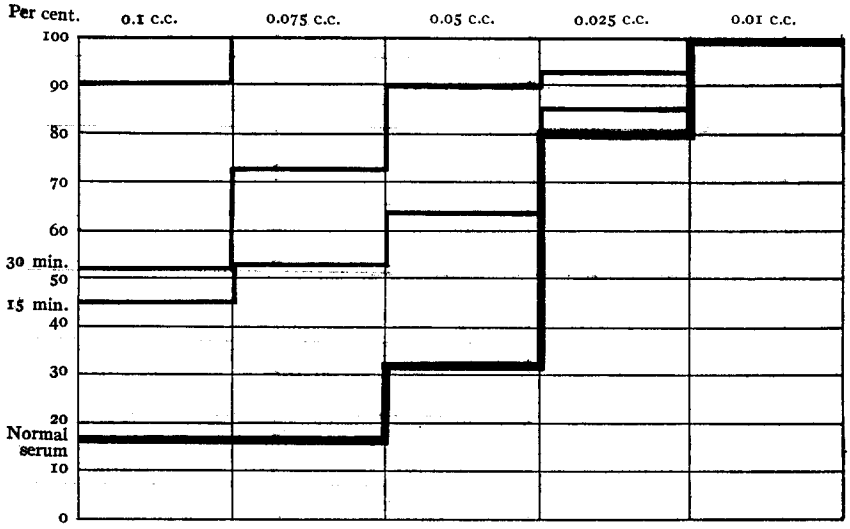
Black line = human serum.
Dotted line = ether-extracted human serum.
Broken line = ether-extracted human serum, ether evaporated.

TEXT-FIG. 2. Effect of evaporating ether extract and partial reactivation of serum.

of the serum restored. The ether was removed from the second tube by means of a pipette, and the serum was then filtered several times through filter paper. It was made neutral by adding a dilute solution of sodium hydrate. Text-figure 2 shows the results obtained in this experiment. The chart shows that only 8 per cent.

of digestion was obtained in the flask containing 0.2 of a cubic centimeter of the untreated serum, while 100 per cent. of digestion was obtained in the flask containing the same amount of ether-extracted serum. The serum which had been made slightly alkaline after evaporation of the ether showed about half the inhibiting action possessed by the one to which nothing had been added. These results indicate that the solvent does not destroy the anti-enzyme.

We must assume that the action of the alkali is due to a combination with some substance soluble in ether, as the alkali did not re-



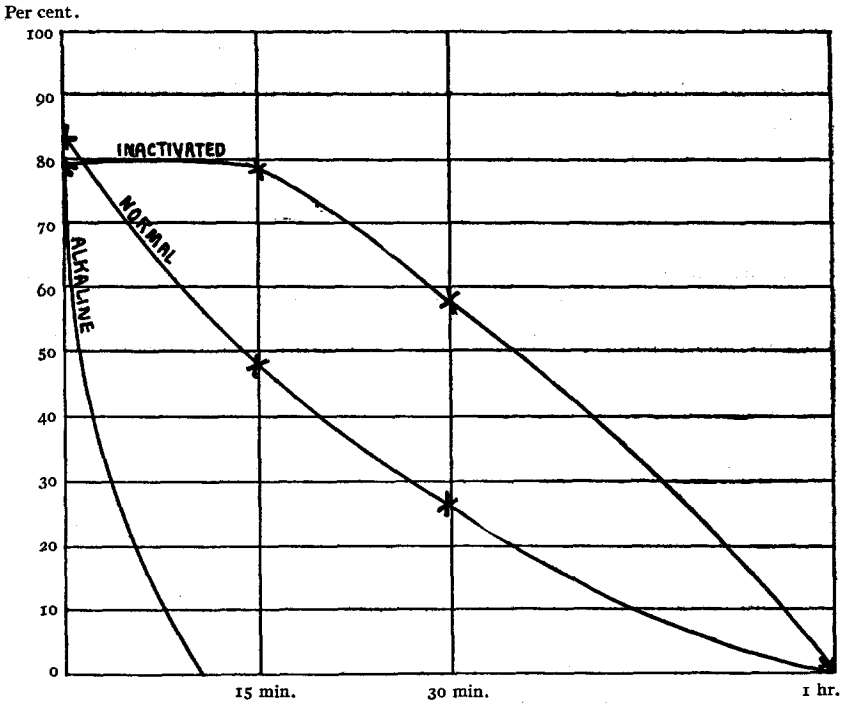
TEXT-FIG. 3. Effect of chloroform on serum antitrypsin at 37° C.

store the activity of the serum from which the ether had been removed, but partially restored it to the serum from which the ether was evaporated. Under these conditions the alkali would not be likely to combine with lipoids such as lecithin, cholesterol, etc., and so only the fatty acids remain. It cannot be expected that the full strength of the inhibiting action observed in the untreated serum should be obtained here. Following the evaporation of the ether, the extractives would be more or less insoluble in the serum and this would render it impossible to saponify all the free fatty acids without the aid of heat and an excess of alkali. If the removal of the inhibiting action at room temperature by chloroform is aided by the action of the serum lipase, a shorter time should be required

by incubating the serum at 37° C., ferments being more active at this temperature.

TIME REQUIRED FOR CHLOROFORM EXTRACTION AT 37° C.

In the next experiment we wished to determine the length of time required for chloroform to remove the ferment-inhibiting action of the serum when the mixture was kept at 37° C. Chloroform was added to fresh serum, thoroughly shaken, and the



TEXT-FIG. 4. Rate of removal of serum antitrypsin in normal, inactivated, and alkalinized serum at 37° C.

mixture was placed in the incubator at 37° C. At fifteen minute intervals some of the serum was removed and tested for its ferment-inhibiting properties. In separating the serum from the chloroform it is necessary to centrifuge the emulsion at high speed for about five or ten minutes, and then to filter it through coarse filter paper several times until the serum is quite clear and all chloroform evapo-

rated. This experiment demonstrates that the ferment-inhibiting action of the serum is almost wholly lost after sixty minutes' incubation with chloroform (text-figure 3).

In the next experiment we wished to determine if lipase was essential to the removal of the inhibiting substances by the chloroform. The serum was divided into three portions. One portion was heated at 54° C. for thirty minutes in order to destroy the lipase; the second portion was made distinctly alkaline in order to prevent the serum from becoming acid; and the third portion was used as a control. Chloroform was added to all three portions and they were then placed in the incubator. A portion of each lot was removed at fifteen minute intervals and tested against trypsin for its anti-enzyme properties (text-figure 4).

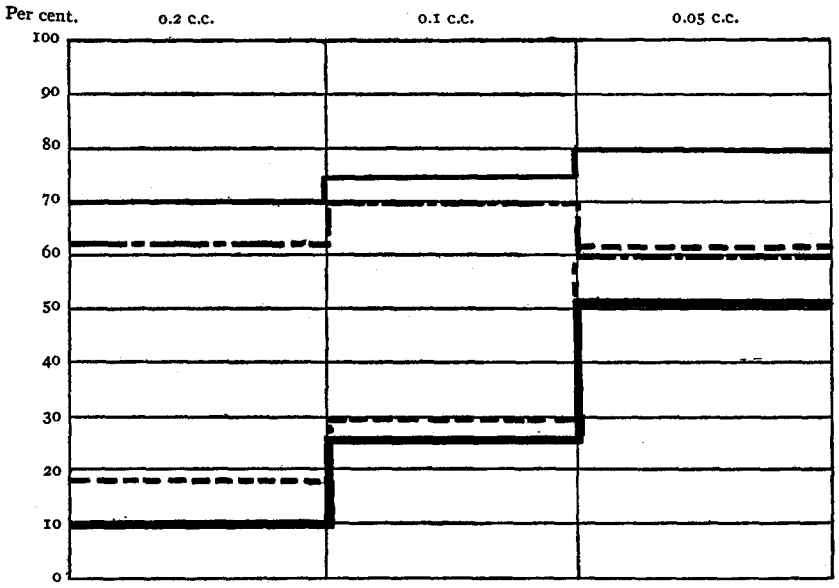
The results show that lipase is not essential to the removal of the inhibiting substances by the chloroform, and indicate that the inhibiting substances are probably fatty acid esters which are soluble in chloroform and ether. There was very little decrease in the inhibiting action of the inactivated serum during the first fifteen minutes of incubation. This may have been due to the absence of the lipase, or to some physical change in the serum caused by heat.

FATTY ACIDS AS ANTIFERMENTS.

The results obtained in the previous experiments indicate that the inhibiting agents are compounds of the fatty acids, and the next experiment was planned to see if this was the case.

Ether was added to fresh dog serum and the mixture was allowed to stand for two days at room temperature. The ether was then removed and divided into two portions. One portion was made slightly alkaline with sodium alcoholate and evaporated to dryness. The soaps were then dissolved in water and filtered; the acids liberated with hydrochloric acid, taken up in ether, and resaponified. The second portion of ether was shaken with water and the ether then evaporated at low temperatures. The soaps prepared from the first portion, the emulsion prepared from the second, and the serum from which the ether extracts were obtained, were tested for their ferment-inhibiting action. The original untreated serum was used

as a control. Text-figure 5 shows the percentage of casein digested by trypsin after it had been incubated for thirty minutes with these preparations. The lines on the chart show that the soaps prepared from the ether extract cause nearly as much inhibition as the untreated serum, while the water emulsion gives 62 per cent. of diges-



Heavy black line = original serum.
Lighter black line = extracted serum.
Dotted line = ether extract saponified.
Broken line = ether extract emulsified.

TEXT-FIG. 5. The antitryptic effect of the ether extract of normal serum when saponified.

tion. The inhibition caused by the emulsion is probably due to some of the acids combining with the alkali which had been used in the preparation of the casein solution. The soaps thus formed would of course inhibit ferment action. The original untreated serum gave 10 per cent. of digestion, and that extracted with ether 70 per cent. Complete removal of the inhibiting action of the serum is rarely obtained in less than four days' extraction at room temperature, and then, as a rule, only with chloroform.

Up to the present time all observers have failed to secure any

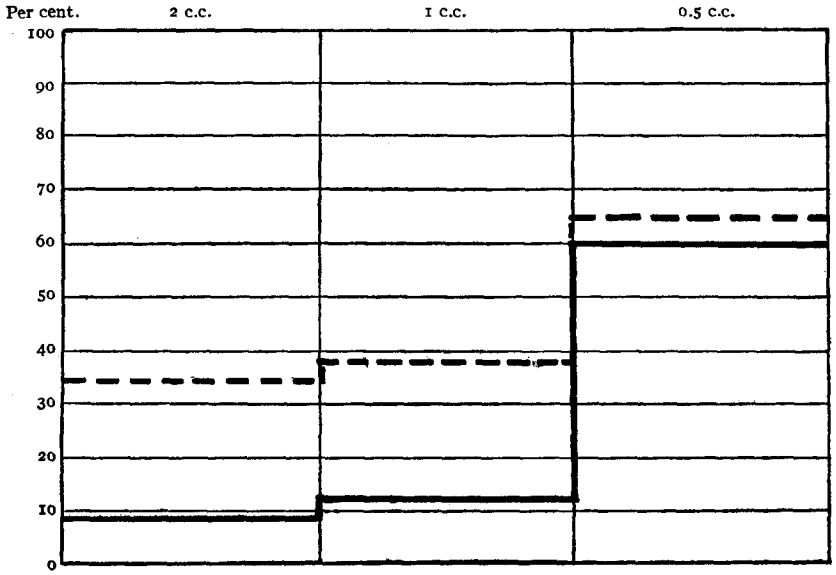
antitryptic effect of the ether or chloroform extracts. This is due solely to the fact that in the simple emulsions with which they worked, the intimate contact of the trypsin and the unsaturated fatty acid complex, which is the basis of the antitryptic activity, is not brought about. In the native serum these lipoids are in a state of dispersion sufficiently great to permit such contact, and we can reproduce it only by saponifying these fatty acid radicals and thus bringing them into a colloidal solution again. We have found that we can at all times secure the antitryptic effect in almost its original value from the extracts, if care is taken to secure complete saponification. If such extracts are not well saponified, so that droplets of unsaponified material can be observed under the high power microscope, then either little or none of the antitryptic effect will be recovered. The results of this experiment indicate that the inhibiting action of the serum is due to the fatty acids. It is not necessary to assume that they can act only as soaps; probably the esters containing unsaturated fatty acids are just as effective.

REMOVAL OF ANTIFERMENT BY FILTERING ACID SERUM THROUGH KAOLIN.

The results that we have been discussing were probably due to the extraction of the fatty acids by the solvent. The fatty acids can also be liberated by means of other acids, in which case they should be thrown out of solution and removed by filtration. The next experiment was planned to find out if the inhibiting substances could be removed by filtering through kaolin serum which had been made strongly acid with hydrochloric acid.

Twenty cubic centimeters of fresh dog serum were diluted with forty cubic centimeters of water, and five cubic centimeters of N/10 hydrochloric acid were added. The mixture was shaken thoroughly, allowed to stand for a few minutes, and then filtered several times through kaolin. After standing for a short time to permit all the serum to drain off, the kaolin was heated with sodium alcoholate. The mixture was then filtered and the filtrate evaporated to dryness. The soaps were dissolved in water and tested for their ferment-inhibiting properties. The acid serum obtained after filtration was made neutral and also tested. Some of the original unacidified

serum was filtered through kaolin to see if kaolin filtration would exert any influence on normal serum. Text-figure 6 shows the results of filtering acidified serum through kaolin. The inhibiting action of the serum which was made acid and then filtered through kaolin was completely lost, and is therefore not shown in the chart. The soaps prepared from the kaolin were active as inhibiting agents



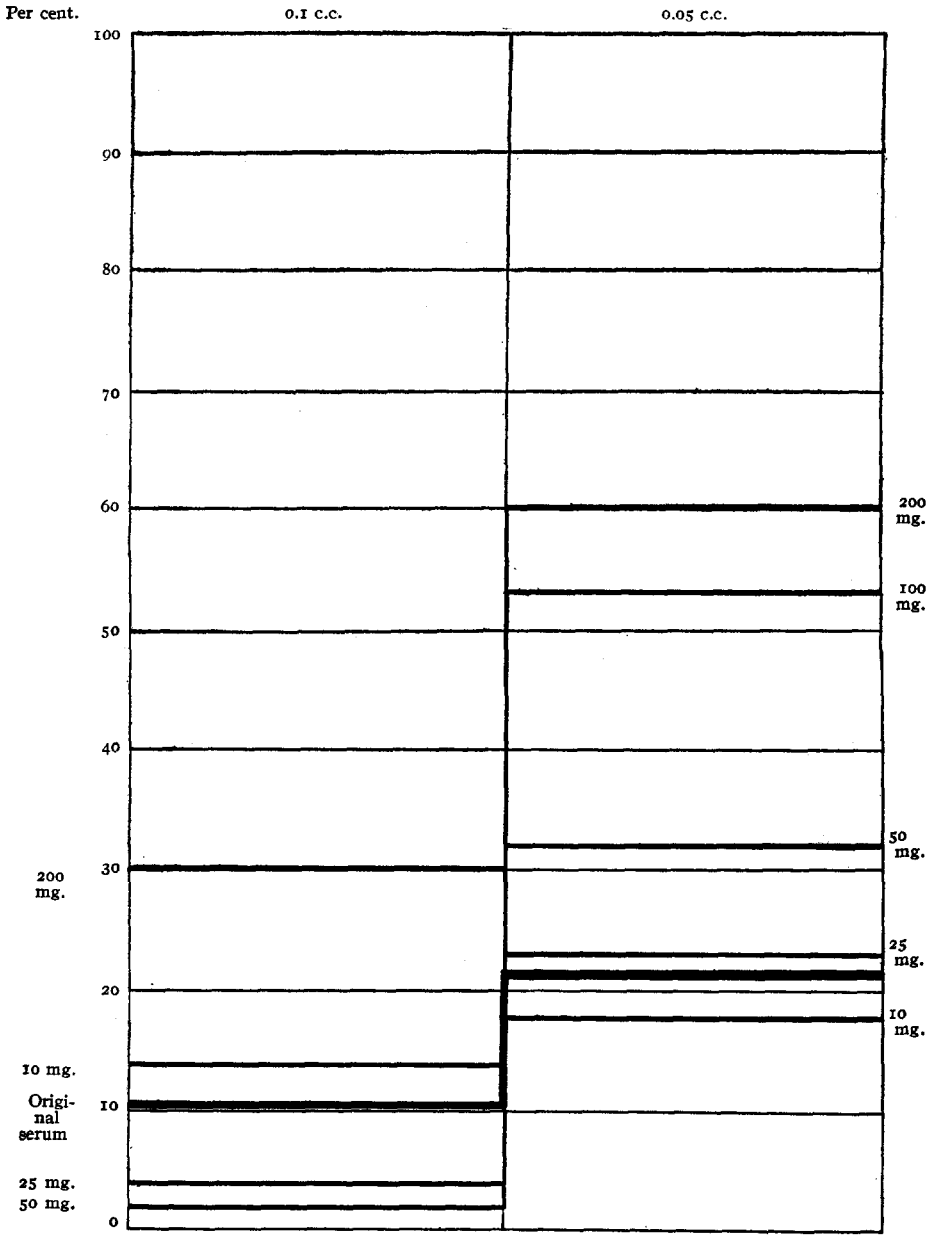
Black line = serum diluted by 10 (horse).
Dotted line = kaolin extract saponified, *i. e.*, $\frac{1}{10}$ strength of serum.

TEXT-FIG. 6. Antitryptic effect of kaolin extract saponified.

and indicate that the fatty acids are the active substances present in the serum. The strength of the soap solution was about 0.1 of that of the untreated serum. We have not been able by this method to recover a larger amount of the inhibiting substances, though the serum has usually lost all its anti-enzyme properties.

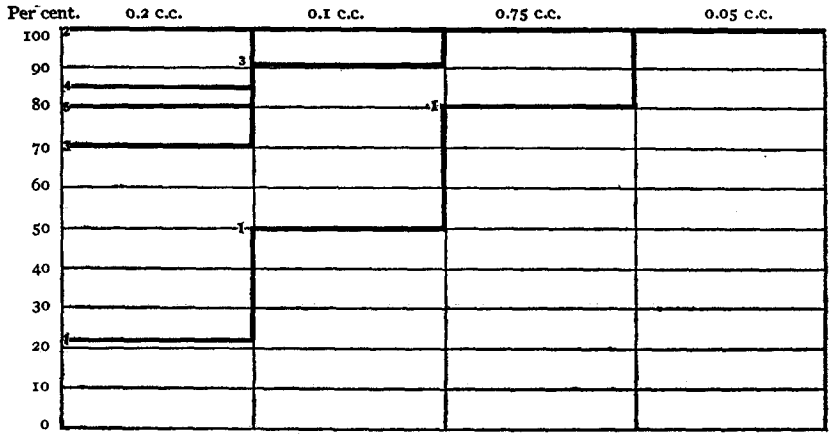
INFLUENCE OF IODIN OR POTASSIUM IODIDE ON ANTIFERMENT.

In our previous work (23) we have shown that soaps of the unsaturated fatty acids lose their ferment-inhibiting properties when treated with iodin. For this purpose we used iodin dissolved in a solution of potassium iodide and removed the excess of iodin by



TEXT-FIG. 7. Effect of varying amounts of potassium iodide on serum antitrypsin.

shaking the mixture with chloroform. This last step was necessary, as iodin inhibits tryptic activity. In the present work we were unable to use the iodin-iodide of potassium mixture, as we have shown that serum loses some of its ferment-inhibiting properties even when shaken with chloroform for a short time. For these reasons we have first investigated the action of potassium iodide alone on the ferment-inhibiting action of the serum.

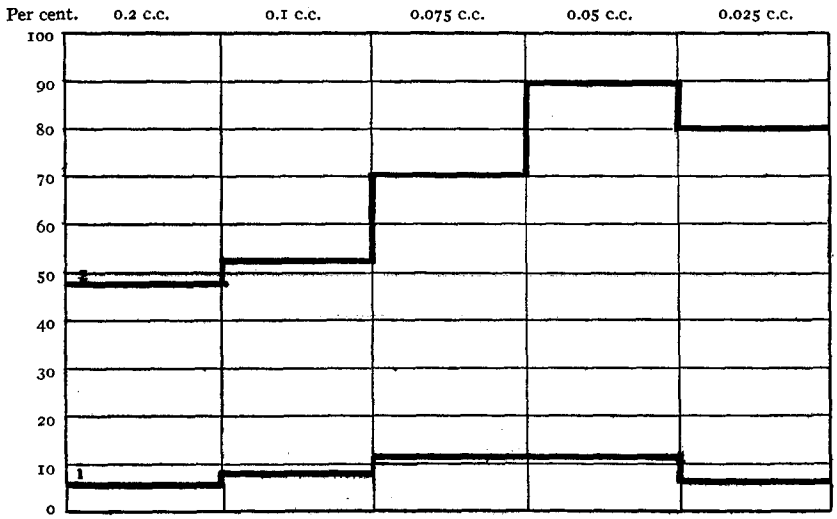


1 = dog serum, 3 days at room temperature.
2 = 1 c.c. dog serum + 200 mg. potassium iodide, 3 days at room temperature.
3 = 1 c.c. dog serum + 100 mg. potassium iodide, 3 days at room temperature.
4 = 1 c.c. dog serum + 200 mg. sodium iodide, 3 days at room temperature.
5 = 1 c.c. dog serum + 100 mg. sodium iodide, 3 days at room temperature.

TEXT-FIG. 8. Effect of sodium iodide and potassium iodide on antitrypsin *in vitro*.

Fresh human serum was divided into six portions of five cubic centimeters each. To one of these portions, potassium iodide was added in the proportion of 10 milligrams to one cubic centimeter; to the second, 25 milligrams to one cubic centimeter; and to the third, 50 milligrams; to the fourth, 100 milligrams; and to the fifth, 200 milligrams. The sixth received no potassium iodide and was used as a control. The tubes containing the sera were placed in the incubator for three hours and afterwards kept at room temperature over night. The following morning they were incubated for thirty minutes with trypsin, and the casein was then added. Control tubes containing trypsin and potassium iodide, without serum, showed

that the chemical did not inhibit the activity of the trypsin. Text-figure 7 shows the influence of potassium iodide on the ferment-inhibiting action of the blood. The chart shows that potassium iodide even under these conditions destroys some of the ferment-inhibiting action of the serum. In the first column in which 0.1 of a cubic centimeter was used, the influence was not marked; but in the second column in which 0.05 of a cubic centimeter of serum was used, there was considerable diminution of the inhibiting action.



1 = guinea pig serum (1 c.c. diluted by 10), normal.
2 = guinea pig serum (1 c.c. + 200 mg. potassium iodide), over night in incubator.

TEXT-FIG. 9. Effect of potassium iodide on antitrypsin of guinea pig serum.

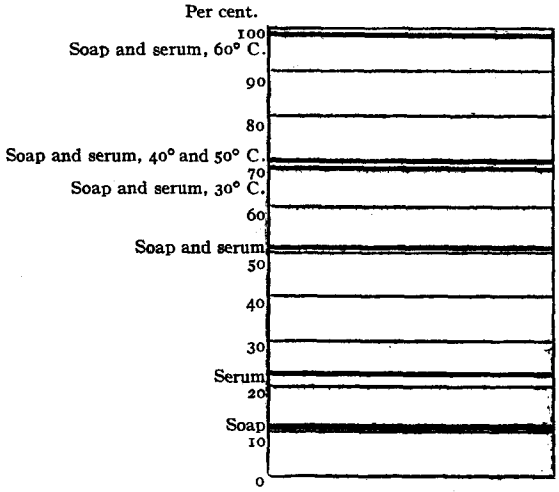
Another experiment was made to see if other iodides would act in a similar manner. In this instance fresh dog serum was treated with sodium iodide and potassium iodide. The technique used was the same as that described for the preceding experiment except that the mixtures were kept for two days at room temperature. The results of this experiment (text-figure 8) are more striking than those of the preceding one. This may be due to the fact that the iodides were in contact with the serum twenty-four hours longer. The sodium iodide is just as active as the potassium iodide in removing the inhibiting action of the serum.

Still more conclusive are the results obtained with guinea pig serum, as this serum is more active as an inhibiting agent. The mixture of serum and potassium iodide stood in the incubator over night. Text-figure 9 shows the influence of potassium iodide on guinea pig serum.

The effect of potassium iodide on the serum antitrypsin seems to be most marked on fresh sera. Treatment of serum with an equal volume of strong solution of hydrogen peroxide, and incubated over night, also causes complete loss of its antitryptic power.

INFLUENCE OF HEAT.

Serum loses its ferment-inhibiting properties if it is heated for thirty minutes at 65° C. If the fatty acids are the inhibiting agents, they must undergo some physical or chemical change which renders them inactive. In the next experiment we wished to determine the



TEXT-FIG. 10. Effect of heat on the antitryptic activity of soap and serum. 1 c.c. contains 0.1 c.c. of serum and 0.01 gm. of soap.

influence of heat on the ferment-inhibiting properties of soap when it is mixed with serum. We had found that soaps dissolved in water did not lose their activity when heated at 100° C., and so it was of interest to determine if they were more susceptible in the presence of serum protein.

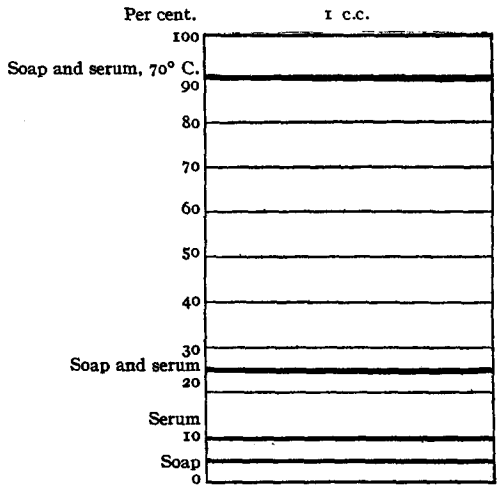
Linseed oil soap, with an iodin value of 110, was used in this experiment. A 10 per cent. solution of dog serum was prepared and to this was added an equal amount of a 1 per cent. soap solution. Portions of the mixture were heated for thirty minutes at 30°, 40°, 50°, and 60° C., and then tested for their ferment-inhibiting properties. The unheated mixture of soap and serum and the unheated soap and serum solutions were tested separately as controls (text-figure 10). The chart shows that the soap solution was active as it permitted only 12 per cent. of digestion, while the serum was less active, permitting 23 per cent. of digestion. The unheated mixture of soap and serum gave 52 per cent. of digestion, while the mixtures heated at 30°, 40°, and 50° C. gave 70 per cent. In the mixture heated at 60° C. the inhibition of ferment action was completely lost.

The amount of inhibition obtained with the unheated mixture of soap and serum was less than that obtained with the soap or the serum alone. This indicated that some of the inhibiting action was destroyed by mixing the serum and soap. In our previous work we found that a slight degree of acidity was sufficient to destroy the activity of the soaps and so we immediately tested the serum used in this experiment. It was found that one cubic centimeter of the serum required 0.1 of a cubic centimeter of an N/10 sodium hydrate solution to neutralize it. This explains why the mixture of soap and serum was less active than the soap or serum separately. The high degree of acidity was probably due to the fact that the serum was several weeks old.

Experiments were then conducted to determine the influence of heat on soap mixed with neutral serum. The same acid serum was made slightly alkaline to phenolphthalein by means of a sodium hydrate solution. Text-figure 11 shows the influence of heat on linseed oil soap when mixed with neutral serum.

Here again the unheated mixture of soap and serum does not inhibit the action of trypsin as much as the soap or the serum alone, though it does show more inhibition than when the serum was acid in reaction. It is possible that here, also, there was some dissociation of the soaps which caused a decrease of inhibiting action. Heating at 70° C. caused almost complete loss of activity of the soap-serum mixture.

Further experiments were made with serum which had been rendered distinctly alkaline by adding 0.1 of a cubic centimeter of N/10 sodium hydrate solution to the neutral serum. This alkaline serum-soap mixture lost only 30 per cent. of its inhibiting proper-



TEXT-FIG. 11. Effect of heat on the antitryptic activity of soap and serum in neutral reaction.

ties when heated for thirty minutes at 70° C. In other words, loss of activity as the result of heating appears to bear some relation to the reaction of the serum.

DISCUSSION.

The results obtained in the preceding experiments justify, we think, the conclusion that the ferment-inhibiting properties of the serum are due to the presence of compounds of the unsaturated fatty acids. Their solubility in ether or chloroform suggests that they are not in the form of soaps, but are present as esters. In some experiments which are not described in this paper, we found that a fine emulsion of olein in serum inactivated by heat, or in salt solution, possessed some ferment-inhibiting action, the inhibiting action bearing a distinct relation to the degree of dispersion. This observation is significant as numerous investigators have reported that the antitrypsin of the serum is increased after eating.

In our study of the ferment-inhibiting substances present in tuberculous caseous material (24) we at first thought that the unsaturated fatty acids were present in the form of soaps, but subsequent experiments showed that they could also be extracted by means of chloroform or ether, after which the material was digestible by trypsin. These results indicate that here also the acids in the form of esters are active as inhibiting agents.

If fresh serum is tested daily for its ferment-inhibiting action it will be found that its strength decreases from 5 to 10 per cent. for the first few days; after that it remains almost constant for an indefinite period of time. With this decrease in anti-enzyme activity there is an increase in acidity. The acidity is probably due to the liberation of fatty acids by the action of the serum lipase on the fatty acid compounds present. If serum lipase is mixed with ethylbutyrate and then incubated, the degree of acidity will be found to be practically the same at the end of four hours as after twenty-four hours. But if the mixture is made neutral at the end of the first four hours and again incubated, the acidity at the end of the second four hours will be almost equal to that obtained after the first incubation. In other words, the ferment can act to a certain point only; after that its action is inhibited either by the amount of acids liberated or by the synthetic compounds formed from the acids. When chloroform or ether is added to the serum, the acids are taken up by the solvent almost as fast as they are liberated, and so they do not interfere with the action of the lipase. Under these conditions the ferment remains active until most of the substrate consisting of the fatty acid compounds has been destroyed. We have found that chloroform acts better than ether in removing the inhibiting agent, and this is to be expected as chloroform is known to exert less influence on ferment activity. This hypothesis also explains why a serum can be left for months with other preservatives without losing all its antiferment properties. In order to remove the inhibiting substances with chloroform it is necessary that a large amount of the solvent be used, and that the mixture be shaken frequently.

Two possibilities must be considered in explaining the destruction of the ferment-inhibiting properties of the serum by heat: first,

it might be due to saturation of the unsaturated fatty acids; second, to the mechanical inclusion of the fatty acid compounds in the fine coagula caused by the heating. If the fatty acids become saturated when heated with protein the combination formed must be easily dissociated, as their activity is not permanently lost. If an inactive soap-serum mixture is saponified with strong solutions of sodium hydrate, and the fatty acids are then liberated in the usual manner and resaponified, it will be found that the soaps are just as active as those obtained from an unheated portion of the same mixture. At first we thought the destruction of the inhibiting action was due to the mechanical inclusion of the fatty acid compounds in the fine coagula caused by heating, but subsequent experiments with sera made strongly alkaline with sodium hydrate disproved this. Under these conditions, coagula would not form, yet the resistance of the inhibiting substances to heat was greatly increased. We have noticed frequently that there is an increase of from 5 to 20 per cent. in the antiferment activity of sera heated for fifteen minutes at 54° to 55° C., especially in sera that are lipemic.

Thiele (25) found that the neutral fat of chyle was adsorbed by serum when the mixture was kept for several hours at 37° C. The fat in such a mixture could not be extracted with ether, but the combination could be broken up by peptic digestion and by treatment with alcohol. A similar explanation may apply to our observation that a soap-serum mixture heated at 70° C. loses its anti-enzyme properties. The iodin values of the heated and unheated mixtures are the same. We must therefore assume that the combination formed is one which is readily dissociated.

The effect of the potassium iodide is probably due to the liberation of small amounts of free iodin which can then saturate the unsaturated carbon bonds. Thus the iodin value (determined by precipitating the serum proteins by alcohol and titrating the alcoholic filtrate) of a serum treated with potassium iodide falls to almost one fifth of the original value. Free iodin dissolved with a small amount of potassium iodide also lowers the antitryptic activity. The relation of the iodide to serum antitrypsin will be discussed in a later paper. The influence of iodin in removing the inhibiting action of the serum shows that the inhibiting agents belong to the unsaturated group of the fatty acids.

The effect of hydrogen peroxide in completely destroying the antitryptic activity; the fact that the extracted lipoids, which are the active agents, are all unsaturated compounds, having an iodin value of from fifty to sixty; and also the fact that sera with a high antitryptic index give a higher iodin value than sera with a low antitryptic index, all tend to confirm this view from a chemical standpoint. From the clinical investigations so far reported there is similar confirmation; there is an increase of the antiferment after eating; in the acute infections such as typhoid fever, pneumonia, etc.; in chronic infections such as syphilis and tuberculosis; in Graves' disease; and in the severe anemias, such as those accompanying malignant growths, etc. In all but the first of these conditions, the demands of the body or the action of the toxins, cause a marked inroad into the fat depots of the body. This disappearance of fat in conjunction with a lowering of the oxidation powers of the liver and tissues in general causes an increase in the amount of unsaturated fatty acids in the blood and thus an increase in the ferment-inhibiting action.

CONCLUSIONS.

1. The ferment-inhibiting action of the serum is due to the presence of compounds of the unsaturated fatty acids.

2. These fatty acid compounds may be removed from the serum by means of chloroform or ether.

3. Soaps prepared by saponifying the chloroform or ether extracts inhibit the action of trypsin.

4. The anti-enzyme action of the serum can be removed by filtering acid serum through kaolin, and can in part be restored by extracting the kaolin.

5. The decrease in strength of anti-enzyme in old sera is probably due to the action of the serum lipase.

6. Iodin, potassium iodide, or hydrogen peroxide remove the inhibiting action of the serum.

7. Soaps of the unsaturated fatty acids lose their ferment-inhibiting action when heated with serum at 70° C.

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