

SERUM CHANGES FOLLOWING KAOLIN INJECTIONS.

STUDIES ON FERMENT ACTION. XXV.

BY JAMES W. JOBLING, M.D., WILLIAM PETERSEN, M.D., AND
A. A. EGGSTEIN, M.D.

(From the Department of Pathology, Medical Department, Vanderbilt University, Nashville.)

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The observation of Keysser and Wassermann (1), that the serum of certain animals becomes toxic when incubated with an inert substance such as kaolin, has stimulated considerable interest in the mechanism of the reactions that occur. Keysser and Wassermann considered the phenomenon due to an adsorption of amboceptor. Gengou had noted that quite indifferent substances (he used barium sulphate and calcium fluoride) were hemolytic and that this effect was held in abeyance by serum.

Friedberger and Kumagai (2) made similar observations with kaolin and determined that the serum albumin and globulin protected against the hemolytic effect, while the split products of the proteins protected in a degree corresponding to their complexity. They decided that the inhibiting substance of the serum was not lipoidal, for they found that if they washed the serum ten times with ether the serum still protected. They do not explain the loss of the inhibitory effect of the serum, for if this is due solely to protein bodies we can hardly see why single or even repeated treatments with the kaolin should exhaust the serum.

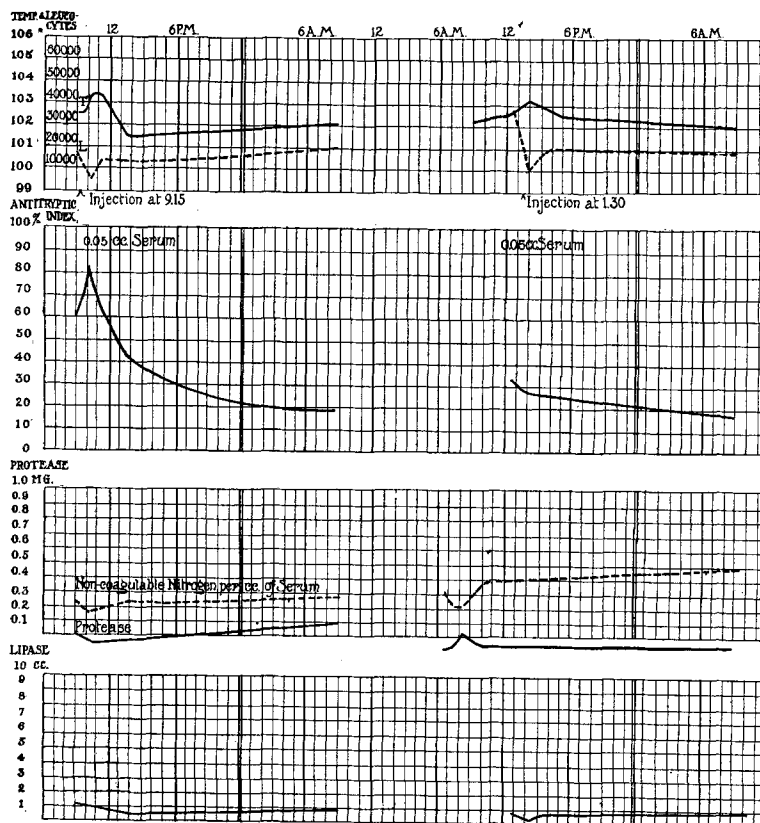
Friedberger at first considered the effects of intravenous injection of inert substances, such as kaolin, as purely mechanical, *i. e.*, plugging of the capillaries of the brain and lung, in this way leading to an acute death. Later, however, together with Tsuneoka (3) he admitted that the toxic effect could not be so interpreted, for after treatment with serum the kaolin was non-toxic.

In previous papers (4) we have developed the idea that it is the serum antiferment which is most readily adsorbed from the serum by inert substances such as kaolin and barium sulphate. By such adsorption local areas of antiferment deficiency are formed where proteolysis can take place, with a resulting production of toxic split products. Inasmuch as this is the mechanism in anaphylatoxin formation we might expect that the intravital injection might give evidences of a similar condition. The following experiments have been carried out with this point in view.

EXPERIMENTAL.

Dog II.—Weight 5.5 kilos. 0.05 gm. of kaolin suspension was injected intravenously at 9.15 a. m. The animal showed evidence of malaise; was nauseated; temperature increased slightly after 1 hour but fell in the afternoon.

From Text-fig. 1 it will be observed that there was at first a sharp rise in the antiferment, followed by a gradual decline. The serum lipase showed a slight decrease, as did also the protease. A second



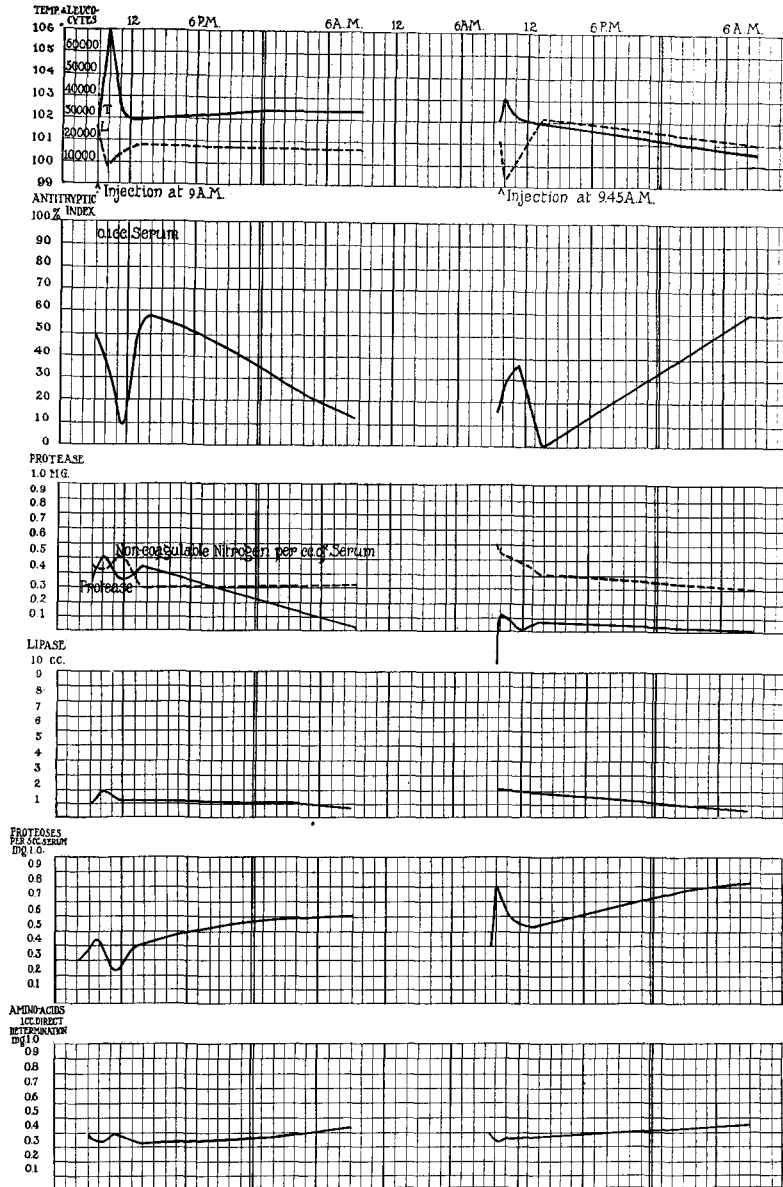
TEXT-FIG. 1.

TEXT-FIG. 1 a.

TEXT-FIGS. 1 AND 1 a. Serum changes following intravenous kaolin injections.

injection was made 4 days later (Text-fig. 1 a), double the amount of the first injection being used (0.1 gm.). The temperature and the leucocytic reaction were similar, although the antiferment

Serum Changes Following Kaolin Injections.



TEXT-FIG. 2.

TEXT-FIG. 2 a.

TEXT-FIGS. 2 AND 2 a. Serum changes following intravenous kaolin injections.

showed less alteration. In the afternoon there was a considerable increase in the non-coagulable nitrogen in the serum. The protease was only temporarily increased.

Dog 66.—Weight 6 kilos. 0.5 gm. of kaolin was injected at 9.45 a. m. (Text-fig. 2 a). This amount caused complete prostration with nausea, vomiting, and diarrhea. The blood taken 15 minutes after the injection remained fluid.

As will be noted from Text-fig. 2 a there was an immediate rise in antiferment, followed by a secondary fall with recovery the following morning. The non-coagulable nitrogen decreased while there was some increase in the protease, which remained low throughout. The lipase showed a gradual decrease. There was an immediate increase in the proteoses in the serum, while at the same time a slight decrease in amino-acids was noted.

Dog 63.—Weight 6.3 kilos. Sensitized Apr. 2, 1915, with horse serum albumin. 0.5 gm. of kaolin was injected at 9 a. m., Apr. 21, 1915.

This animal differed in its response in the greater rise in temperature and an immediate fall in antiferment (Text-fig. 2); indeed the antiferment curve is exactly the reverse of that of Dog 66. There was noted an immediate rise in proteoses, while the amino-acids decreased slightly as in Dog 66.

The following dogs (Table I) received doses which were almost immediately followed by evidence of a profound shock and an early death. In each case the symptoms were identical.

TABLE I.

Dog No.	Weight. kilos	Injection.				Result.
67	5.0	1	gm.	kaolin	in suspension	Died, 10 min.
65	6.2	0.8	"	"	" "	" 20 "
68	6.2	0.6	"	"	" "	" 5 "
69	6.0	0.4	"	"	" "	" 15 "

The blood examinations immediately before the injection and at the time of death are given in Table II.

It will be observed that there is a distinct increase in the amount of protease in the serum, as also of the higher split products, while the amino-acids show practically no change. The antiferment titer is markedly increased.

TABLE II.

Dog No.	Time.	Total non-coagulable nitrogen per cc.	Protease action per cc.	Proteoses per 5 cc. of serum.	Amino-acids per cc. (direct determination).	Lipase per cc.	Antiferment inhibition.
		mg.	mg.	mg.	mg.	cc. N/100NaOH	
67	Before injection	0.28	0.22	0.5	0.2	0.5	17% per 0.1 cc.
	After "	0.32	0.30	0.74	0.30	0.2	50% " " "
65	Before "	0.28	0.04	0.45	0.41	2.2	33% " 0.2 "
	After "	0.28	0.37	0.41	0.42	2.2	75% " " "
69	Before "	0.31	0.37	0.55			
	After "	0.32	0.68	0.83			

DISCUSSION.

The experiments which have been detailed above give evidence that the toxicity of an inert substance such as kaolin depends on adsorption phenomena which result in protein splitting. Friedberger and Tsuneoka consider that an adsorption of certain substances from cells essential to life takes place. If that were the case we should expect that the serum would protect against the toxic effect, or, if this protection were not sufficient, that the red cells would be the first to be injured with a resulting hemolysis. This, however, never takes place *in vivo*, even during the acute shock. On the other hand, the shock bears much resemblance to the intoxication brought about by protein split products in its effect on coagulation, gastrointestinal intoxication, the immediate leucopenia, etc. If this is the case we should expect to find an increase in protein split products in the serum and an increase in the serum protease.

That this is actually the case will be observed from the text-figures and Table II. The only difference to be noted is the absence of any rise in the serum lipase which follows shock in anaphylaxis and pepsin poisoning. If the adsorption that occurs when the kaolin reacts with the serum *in vitro* or *in vivo* were one of proteins, as Friedberger suggests, there would be no occasion for protein splitting. If, on the other hand, an adsorption of antiferment occurs (the antiferment being lipoidal is adsorbed most readily from the serum), we can easily understand that wherever such adsorption is in progress a localized area of antiferment deficiency must for the moment exist in which splitting can occur whenever ferments are present.

Just as in anaphylatoxin formation the matrix of the split products is to be found in the serum proteins, the kaolin acts merely as an agent which for an instant alters the normal ferment-antiferment balance and in this way brings about a protein intoxication.

Considering the fact that the first effect of acute shock is an increase of proteoses with practically no effect on the amino-acids, it would seem probable that the splitting takes place only to the higher stages. The splitting evidently differs from that occurring during anaphylactic shock (5), when we find first a decrease in proteoses and an increase in amino-acids, indicating a splitting through the peptone stage.

Whether other formed bodies, such as bacteria, ever bring about a toxic effect in this manner is of interest rather from a theoretical than a practical point of view, for the number of bacteria present in the serum would have to be very great to bring about marked changes. Bacteria do, however, readily adsorb the serum lipoids (6), and it seems probable that many of the phenomena which we classify under "*Serumfestigkeit*" are due to the changes so brought about.

CONCLUSIONS.

1. The intoxication produced by the intravenous injection of inert substances such as kaolin is due to protein split products derived from the serum proteins.
2. The kaolin acts as an adsorbing medium for the serum anti-ferment, bringing about an alteration in the ferment-antiferment balance.
3. The intoxication is accompanied by an increase in serum protease, and of proteoses.
4. The serum lipase, the amino-acids, and the total non-coagulable nitrogen show relatively little change.
5. The antiferment shows an initial increase, followed by a loss.

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