LVII. ON THE BEHAVIOUR OF AMYLASE IN THE PRESENCE OF A SPECIFIC PRECIPITATE.

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The complement-binding phenomenon which takes place in the presence of antigen and antibody has become of great practical importance in the recognition of disease. The difficulties of the test are great, owing to the fact that five different, chemically impure, and little known substances must be employed: antigen, antiserum, complement, amboceptor, and red corpuscles. The last three components are very troublesome to obtain, and it is not surprising that many efforts should be made to minimise this trouble, and to lessen the difficulties which are necessarily dependent on such a complicated process. The most attractive simplification would be one in which all these three components of the haemolytic system, viz. complement, amboceptor, and red corpuscles, are entirely done away with. This would be possible if some substance could be found which would become fixed, like complement, by the antigen-antibody mixture, but whose absence could be tested for more easily.

Like complement, animal ferments seem to be very rapidly absorbed by coagulated colloids and even by organic precipitates of different kinds. The ferment shows some selective action in this tendency. Pepsin absorbed into egg-white [Dauwe, 1905, p. 426] or pepsin, ptyalin, etc. into collodion [Porter, 1910, p. 382] can be recovered in some degree by a solution of the particular substance which they digest. As ferments can only be recognised through their effects, this possibility of partial recovery presents the chief objection to the use of ferments as substitutes for complement.

Most ferments are powerfully influenced by the presence of serum. Proteolytic ferments are inhibited by serum, probably because their attraction to the serum proteins, even in a dissolved condition, is already so great. On the other hand other ferments, such as starch-splitting ferments and lipases, are markedly accelerated by the presence of serum.

Hailer [1908, p. 280] first made the attempt of absorbing a ferment by means of a specific precipitate. He chose rennet, with sheep's serum as antigen, and the serum of a rabbit immunised against sheep's protein as antibody. This choice was perhaps unfortunate, as not only rennet but also antirennet have been described in serum [Fuld and Spiro, 1900, p. 141]. Also, as I have pointed out, rennet suffers very little from dilution, when the dilution has been freshly made. Rennet has indeed acted perfectly in my hands [1911, p. 394] at a dilution of 1/340,000. It can therefore be almost entirely absorbed without any evidence of the fact. On this account I thought it advisable to attempt the absorption with another ferment. Pepsin and trypsin are inactivated by serum; I therefore chose amylase. Szumowski [1898, p. 162] has proved that amylase is absorbed by fibrin. It is much less easily absorbed by serum proteins in their natural state, as serum is itself amylolytic. The advantages of the ferment were therefore as follows:

- (1) It is present in serum, so that, were the absorption successful, no further addition of ferment to the antiserum would be required.
- (2) It is apparently not absorbed by serum proteins in their normal state, i.e. before the union of antigen and antibody.
- (3) It loses rapidly on dilution, so that even a small loss could be measured.

METHOD.

Ferment, guinea-pig-serum, saliva, and taka-diastase.

Antibody, serum of rabbits immunised against egg-white or against horse-serum.

Antigen, egg-white or horse-serum.

The ferment, in as small bulk as possible, i.e. 0.05 cc. saliva, or 0.15 cc. diluted taka-diastase, was added to 0.15 cc. immune or normal serum, and 0.15 cc. of the various dilutions of egg-white or horse-serum, the mixture being left at room temperature overnight. Next morning 3 cc. of a 1% soluble starch solution were added, and the tubes were placed at 37° for 5 to 20 minutes, or in the case of guinea-pig-serum for a half to one and a half hours. The mixtures were then tested for sugar by means of Fehling's solution, and for dextrans by means of iodine. On account of the protein present, use was made of the dialysed iron method, which had the double advantage of removing both protein and ferment. When the mixtures were taken from the incubator, they were made up to a bulk of 5 or 6 cc. with physiological saline solution, and 0.5 cc. saturated salt solution, and 0.5 cc.

B.P. Liquor Ferri Dialysatus were added to each and the resulting precipitate immediately filtered off. A measured amount of the perfectly clear and inactive filtrate was then tested quantitatively for sugar. The colour given by the iodine test in the filtrate was clear and permanent.

The difference between the effect of immune and normal serum on amylase in the presence of antigen was very slight, but on account of the accuracy of the method and the number of times the experiment has been repeated, it may be taken as the expression of a genuine though very partial absorption.

Absorption experiment.

A. Immune serum. Rabbit immunised against egg-white.

(To each tube were added 0.05 cc. saliva, 0.15 cc. anti-egg-serum, 0.15 cc. diluted egg-white, method as above.)

Dilution of egg-white	Time at 37° with starch	Co. of Fehling sol.	Iodine solution 0.5 cc.
1/10	10 minutes	0.2	Dark mauve.
1/50	"	0.225	"
1/100	"	0.225	"
1/250	"	0.25	"
1/500	"	0.25	Light mauve.
NaCl	"	0.3	Pale pink.
1/10	20 minutes	0.25	Dark mauve.
1/50	"	0.25	Medium mauve.
1/100	"	0.25	"
1/250	"	0.275	Medium light mauve.
1/500	"	0.3	Light mauve.
NaCl	"	0.35	Very pale.

B. Normal rabbit-serum.

(To each tube were added 0.05 cc. saliva, 0.15 cc. normal serum, 0.15 cc. diluted egg-white, method as above.)

1/10 to 1/500	10 minutes	0.3	All pale.
NaCl	"	0.3	Pale.
1/10 to 1/500	20 minutes	0.375	"
NaCl	"	0.375	"

When serum is used as antigen a curious phenomenon may be noticed. In this case all three ingredients, antigen, antibody, and saliva, contain amylase from three separate species, and this gives rise to an acceleration which is decidedly beyond the sum of the three separate activities. Although the antiserum was heated at 56° to reduce its amylolytic power, it was still able to exert an accelerating influence. In the following experiment, where the antigen is horse-serum, acceleration is to be seen and must be allowed for. In spite of it, absorption can be observed if the "immune" and "normal" columns are compared.

A. *Rabbit-serum*, immune to horse-serum.

(To each tube were added 0·05 cc. saliva, 0·15 cc. anti-horse-serum, 0·15 cc. diluted horse-serum, method as above.)

Dilution of horse-serum	Time at 37° with starch	Ccs of Fehling sol.	Colouration with iodine solution
1/10	10 minutes	0·5	Red mauve.
1/50	"	0·45	"
1/100	"	0·4	"
1/250	"	0·4	Same, but deeper.
1/500	"	0·35	Mauve.
NaCl	"	0·35	"
1/10	20 minutes	0·5	Pale pink.
1/50	"	0·5	"
1/100	"	0·45	"
1/250	"	0·4	Mauve.
1/500	"	0·35	"
NaCl	"	0·35	"

B. *Normal rabbit-serum*.

(To each tube were added 0·05 cc. saliva, 0·15 cc. normal rabbit-serum, 0·15 cc. diluted horse-serum, method as above.)

1/10	10 minutes	0·55	Colourless.
1/50	"	0·55	"
1/100	"	0·5	"
1/250	"	0·4	Pale.
1/500	"	0·35	Mauve.
NaCl	"	0·35	"
1/10	20 minutes	0·6	Colourless.
1/50	"	0·6	"
1/100	"	0·55	"
1/250	"	0·45	"
1/500	"	0·4	Pale.
NaCl	"	0·35	Mauve.

Taka-diastase displayed no tendency whatsoever to become absorbed by a specific precipitate. This is interesting, as I have noticed before [1910, p. 386] that while ptyalin was inactivated by collodion membranes in a day, taka-diastase was hardly affected in a month's time.

BEHAVIOUR OF SERUM AMYLASE ON PRECIPITATION WITH CARBON DIOXIDE.

Fresh guinea-pig-serum, diluted to 1/10 in water, was saturated with carbon dioxide, and the globulins separated and redissolved in saline solution, after centrifuging. The upper fluid taken from the centrifuge had its isotonicity restored by sodium chloride, and both portions were tested for amylase. The ferment was found to be almost unaffected by the precipitation, practically the whole ferment remaining free in the upper fluid.

Time $1\frac{1}{2}$ hours	Cc. of Fehling sol.	Colouration with iodine
1 cc. serum 1/10 + 1 cc. NaCl 0·85 %	0·5	Brown.
1 cc. precipitate (1/10) + 1 cc. NaCl 0·85 %	0·05	Dull, dark.
1 cc. upper fluid + 1 cc. NaCl 0·85 %	0·4	Brown.
1 cc. serum 1/10 + 1 cc. precipitate	0·475	„
1 cc. serum 1/10 + 1 cc. upper fluid	0·65	Colourless.
1 cc. precipitate + 1 cc. upper fluid	0·425	Brown.

Although these experiments have been attended with little success, I am venturing to record them as of some small theoretic interest, and partly to recommend a good method for testing amyolytic action in the presence of serum.

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