CXL. ON THE INVERTASE-ACCELERATOR OF SERUM, WITH OBSERVATIONS ON OTHER ENZYME-ACCELERATORS REPORTED IN SERUM.

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(Received May 15th, 1930.)

THE phenomenon of invertase-acceleration by serum was first observed in the course of a biochemical investigation into the causal factors of an experimental immunity produced against the myiasis-producing larva of *Cordylobia anthropophaga* [Blacklock, Gordon and Fine, 1930]. Although this phenomenon was of no interest from the point of view of the inquiry that led to its discovery, I became interested in it because of its possible application to diagnosis.

Further investigation into the properties of the invertase-accelerator, however, revealed the fact that acceleration could be replaced by inhibition by altering the p_H of the enzyme mixtures.

Throughout the investigations vegetable invertase only was used; no studies were carried out with animal invertase.

HISTORICAL.

The term "enzyme-accelerator."

It is necessary to point out that this term is not synonymous with the term co-enzyme, which nevertheless has been used as an alternative name for it [Lewis and Corran, 1928]. A co-enzyme is an integral part of the enzyme system itself, which cannot function apart from it. An accelerator, however, merely increases the velocity of the reaction produced by the enzyme.

Unfortunately, the term co-enzyme was first applied [Bertrand, 1897], to a substance which was really an accelerator—namely, to manganese in its action on laccase.

One of the first examples of a true co-enzyme was supplied by Magnus [1904], who proved that liver extract lost its lipolytic action on dialysis: the dialysate, which restores lipolytic action on addition to the residue, is the lipase co-enzyme.

Enzyme-accelerators in serum.

The literature contains references to three enzyme-accelerators in serum, namely, lipase-accelerator, amylase-accelerator, and invertase-accelerator. When serum also contains the corresponding enzyme, it must obviously be a matter of some difficulty to prove the existence of an accelerator as well, and I propose to show that the evidence submitted for the existence of an amylase-accelerator is inadequate.

Examination of the evidence for amylase-accelerator. It has been shown repeatedly, since Magendie's first demonstration [1846], that serum possesses diastatic action. Consequently the addition of serum to an active diastatic solution would enhance the action of the latter merely by virtue of the presence of diastase in serum. Such enhanced action in itself is no evidence of the presence of an accelerator. Thus, the work of Pozerski [1903], Wohlgemuth [1911], and Crohn and Epstein [1914], who all showed that serum "stimulates" pancreatic diastase, goes no further than confirming the presence of diastase in serum.

O'Donovan and Davison [1927], however, claim that serum possesses an amylase-accelerator on the ground that the addition of serum increases the starch-liquefying properties of pancreatic extract, while serum alone has no liquefying action on starch. This work, however, merely shows that by using viscometric methods it is impossible to demonstrate amylase in serum. Were it possible to accept the fact that serum does not contain amylase, the inference of the above authors that serum contains an amylase-accelerator would be valid. There is overwhelming evidence however that serum contains amylase.

Lipase-accelerator in serum. This was first demonstrated by Beitzke and Neuberg [1906], and later by Rosenheim and Shaw-Mackenzie [1910]. Some recent workers, however, seem to have overlooked the fact that serum also contains lipase. Serum-lipase has in fact been extensively studied for diagnostic possibilities by Vanysek and Felklova [1924], Katsch [1925], Buchler [1924], and others.

Lipase-accelerator, it has been claimed by Lewis and Corran [1928], is diminished in cancer and restored to normal in the course of lead treatment. These workers, however, entirely ignore the existence of serum-lipase and attribute all action in their serum-lipase mixtures to the accelerating action of serum. Furthermore, they at times refer to their lipase-accelerator as a lipase co-enzyme and quite unnecessarily coin the term "augmentor" for an accelerating substance which has little destructive action on the enzyme. Confusion of this kind as to co-enzyme and accelerator, as well as failure to control the enzyme when investigating enzyme-accelerators, renders invalid a good deal of work on this subject. Not all sera contain lipase-accelerator, though all sera examined so far have been found to contain lipase.

The following table shows the distribution of lipase and lipase-accelerator in some of the sera I examined.

	Lipase	Lipase- accelerator
Guinea-pig	+	-
Rat	+	-
Cow	+	+
Man	+	+
+ = present.		- = absent.

The work of Abderhalden on serum-invertase. In spite of the statement, which is very widespread, that normal serum has the power of inverting sucrose, experiments carried out to test this power in no case confirmed the presence of invertase in serum. The authority for this statement appears to be two papers published by Abderhalden, one in collaboration with Kopfberger [1910], and the second with Schiff [1910].

In the first paper it is claimed that invertase was found in serum by studying the polarimetric changes when serum is mixed with sucrose. In the second paper it is claimed that within a few minutes of injecting sucrose parenterally into a dog there appeared a great increase in the inverting power of serum.

Although Weinland [1907], previously claimed to have obtained serum-invertase following injections of sucrose, no confirmation of these results by other workers could be found; it was therefore considered desirable to test their validity.

EXPERIMENTAL.

Investigation of the results of Abderhalden.

(1) *Examination of serum for invertase.* Two tubes were incubated at 37°, the first containing guinea-pig-serum, sucrose, and buffer, the second, serum and buffer only.

In this, and in all subsequent experiments wherever buffer solutions were required, the buffer solutions of Clark and Lubs were used (acid potassium phthalate or acid potassium phosphate, as required, combined with HCl or NaOH). The composition of the two tubes is indicated in the following table:

	Tube 1	Tube 2
	cc.	cc.
Serum	0.1	0.1
Sucrose 5 %	0.2	—
Buffer p_H 4	0.7	0.9

After 24 hours 0.1 cc. was removed from each tube, and the reducing sugar estimated by a modification of MacLean's method. The composition of the copper solution used was:

CuSO ₄ , 5H ₂ O	...	...	5 g.
Tartaric acid	...	...	14 g.
Na ₂ CO ₃	...	...	130 g.
KI	...	...	5.5 g.
KIO ₃	...	...	0.5 g.
H ₂ O	...	...	to 1 litre.

2 cc. of this fluid were equivalent to 1.2 mg. glucose or 1.2 mg. invert sugar: this amount of sugar just exceeds the maximum obtainable by inversion of the sucrose dissolved in 0.1 cc. of the incubated fluid (*i.e.* 1.05 mg. invert sugar).

The actual estimation was carried out as follows. 0.1 cc. of the incubated fluid was transferred to a test tube and 0.5 cc. phosphotungstic acid mixture (formula by Taervert [1925]) added to coagulate proteins. The mixture was diluted with 5 cc. distilled water, filtered into a boiling-tube, and the residue washed with 5 cc. more distilled water. The filtrate was then heated with 2 cc. of the copper solution for 10 min. in a boiling water-bath. The tube was finally cooled, 1 cc. 25 % HCl was added and the liberated iodine estimated as in the MacLean method, *N*/250 sodium thiosulphate being used. A blank estimation was carried out without incubated fluid.

Although a source of error might be expected from the glucose normally present in serum, this error was found to be negligible in the above experiments.

No reducing sugar was obtained in either of the incubated tubes, even after three days.

This experiment was repeatedly performed, and consistent results were obtained. The sera of cow, rat, sheep, and man gave similar results. A similar experiment carried out with human serum acting at p_H 7 showed absence of any inversion at this hydrogen ion concentration (the optimum for animal invertase).

(2) *Influence of sucrose injections on serum.* Five guinea-pigs were injected intraperitoneally with varying amounts of sucrose. The sera were examined for invertase and sucrose (and in some cases reducing sugars), and the urine was examined for sucrose and reducing sugars.

(a) In the case of serum, tubes were incubated as follows:

	Tube 1	Tube 2	Tube 3
	cc.	cc.	cc.
Serum	0.1	0.1	0.1
Invertase	0.2	—	—
Sucrose 5 %	—	0.2	—
Buffer p_H 5	0.7	0.7	0.9

The invertase used was a commercial preparation (Martindale's) in solution form and preserved in glycerol.

After 24 hours the reducing sugar in each tube was estimated by the modified MacLean's method already described.

Tube 1 indicated the amount of blood-sucrose.

Tube 2 indicated the presence of invertase in serum.

Tube 3 indicated the amount of reducing sugar in serum only if present in increased amount (above 0.4 %) since, as already pointed out, the normal glucose content of serum is inappreciable in the technique adopted. Such increase in the reducing sugar content of serum might indicate that inversion of absorbed sucrose was taking place.

Table I.

Guinea-pig E.

No. of injection	Date of injection	Amount of sucrose injected 5 g. in 12 cc. H ₂ O	Serum			When examined (hrs after injection)	Urine			Fate of guinea-pig Died 17 ii. 29
			Sucrose %	Invertase +=present 0=absent	Reducing sugar		Total period collected (hrs after injection)	Sucrose recovered (g.)	Reducing sugar (as glucose)	
1	28 i. 29	5 g. in 12 cc. H ₂ O	0	0	No increase	3	2	0.36	0	
			0	0	"	19				
			0	0	"	26				
			0	0	"	45				
			0	0	"	50				
2	15 ii. 29	3 g. in 5 cc.	(Serum not examined)				24	0.49	0	
<i>Guinea-pig F.</i>										
1	28 i. 29	1 g. in 5 cc.	0	0	"	4	1½	0	0	Survived
			0	0	"	19				
2	29 i. 29	1 g. in 5 cc.	0	0	"	6	6½	0.13	0	
			0	0	"	25				
3	30 i. 29	1 g. in 5 cc.	0	0	"	5	3¾	0.40	0	
			0	0	"	20				
4	31 i. 29	1 g. in 5 cc.	0	0	"	7	5½	0	0	
			0	0	"	24				
<i>Guinea-pig G.</i>										
1	15 ii. 29	3 g. in 5 cc.	2.5	0	"	Just after death	1½	0.108	0	Died 3½ hr. after injection. Peritoneal fluid, 27 cc. Contained 3 % sucrose
<i>Guinea-pig L.</i>										
1	15 ii. 29	3 g. in 5 cc.	1.7	0	"	Just after death	1½	0.07	0	Died 3½ hr. after injection. Peritoneal fluid=31 cc. Sucrose=2.5 %
<i>Guinea-pig S.</i>										
1	18 i. 29	1 g. in 5 cc.	0	0	"	25 min.	1½	Trace	Trace	Died 16 ii. 29. Peritoneal fluid=9.8 cc. Contained no sucrose
			0	0	"	2	(Amount of urine not measured)			
			4	0	"	19				
			0	0	"	44				
2	13 ii. 29	2 g. in 1½ cc.	0	0	"	1½	24	0.30	0.32	
			0	0	"	5½				
			0	0	"	25				
3	14 ii. 29	2 g. in 2 cc.	0.33	0	"	4½	19	0.32	Trace	
4	15 ii. 29	3 g. in 5 cc.	(Serum not examined)				7	1.0	0	

(b) The urines (collected separately after each act of micturition) were incubated as follows:

	Tube 1	Tube 2
	cc.	cc.
Urine	0.1	0.1
Invertase	0.2	—
Buffer p_H 5	0.7	0.9

The results of the experiments are shown in Table I.

Discussion of foregoing results.

(a) It will be seen that one consistent result obtained is the absence of any inverting power of serum following sucrose injections.

(b) In most cases the injections were followed by excretion of sucrose within an hour. Owing to the difficulties of collecting urine, only limited amounts were collected—usually the quantities passed during the first few hours after injections. Only occasionally was reducing sugar present in the urine, and then in very small amount.

(c) Sucrose was rarely found in the blood except when the injections caused death. Guinea-pig *S* is interesting in showing 4 % sucrose in the serum 19 hours after the first injection, and 0.33 % $4\frac{1}{2}$ hours after the third: yet the serum of *S* never showed a rise in reducing sugar.

(d) Although the five guinea-pigs reacted differently to the injections of sucrose as regards rapidity of sucrose excretion and blood-sucrose content, yet the results seem to justify the conclusion that the normal mode in which the body deals with parenteral introduction of sucrose is not by the formation in the blood of invertase, but simply by excretion of the unchanged sugar.

It is true that not all the sucrose injected was recovered from the urine: but even assuming that the sucrose was not all dealt with by excretion, it is significant that the urine rarely showed the presence of any reducing sugars, while the serum never showed a rise in reducing power. These facts strongly suggest that no inversion took place in the blood. Above all, on no occasion in the course of 23 tests was invertase found in the serum following injections of sucrose; although the tests were carried out at intervals after an injection of 25 minutes to 4 weeks (in the case of guinea-pig *S*).

The question as to whether substrates can act as antigens and evoke the response of enzymes (antibodies) has been much debated [Bayliss, 1923]; the foregoing experiments do not bear out the immunological analogy.

The experimental production of invertase in guinea-pig serum.

Although serum does not normally contain invertase, it can be experimentally endowed with inverting properties.

Intraperitoneal injections of invertase were given to determine whether an anti-invertase would appear in response to invertase injections: the same commercial preparation already referred to was used. It was in the course of these experiments that the observation was made that normal serum acceler-

ated invertase action: this accelerating effect was not affected by invertase injections. The injections, however, conferred on the serum inverting properties, so that, following the injections, the serum contained both invertase and invertase-accelerator.

Two guinea-pigs were injected with invertase, the first receiving a single large injection (5 cc.), and the second receiving 6 successive injections of 1 cc.

The results are graphically represented in Fig. 1.

Fig. 1. Activity of serum-invertase following injection of commercial yeast invertase (Martindale's).

— Guinea-pig A. - - - - - Guinea-pig B.

In each case the serum was examined by incubating 0.1 cc. with 0.2 cc. 5 % sucrose, and 0.7 cc. buffer p_H 4. After 2 hours, 0.2 cc. fluid was withdrawn and analysed for reducing sugar. Controls were throughout incubated containing serum and buffer only; they never gave any reduction.

Abderhalden and Wertheimer [1922] also report failure to obtain anti-invertase following injections of invertase.

Demonstration of invertase-accelerator in serum.

The influence of guinea-pig serum on yeast invertase was determined by incubating the following tubes:

	Tube 1	Tube 2	Tube 3
	cc.	cc.	cc.
Serum	0.2	0.2	—
Invertase 5 %	0.5	—	0.5
Sucrose 5 %	0.2	—	0.2
Buffer p_H 4	1.1	1.8	1.3

The tubes were incubated at 37°, and after varying intervals 0.2 cc. ($\equiv$ 1 mg. sugar) was analysed for reducing sugar.

Results:

No. of tube	Contents	% sucrose inverted in 50 min.	% sucrose inverted in 7 hr.
1	Serum + invertase	100	100
2	Serum	0	0
3	Invertase	35	100

This result was frequently confirmed; in some instances more frequent estimations were carried out than in the above experiment.

Properties of the invertase-accelerator.

Influence of the concentration of invertase. It was found that acceleration was not obtained with all concentrations of invertase. In the foregoing experiment the concentration of invertase used was 1.25 %: greater acceleration was obtained using weaker invertase, but no acceleration was obtained with 5 % or stronger invertase. These results are summarised in Table II.

Table II.

	0.25 % invertase	1.25 % invertase	5 % invertase
Action without serum (A) (% inversion in 1 hr.)	1	42	No acceleration by serum obtained
Action with serum (B) (% inversion in 1 hr.)	30	100	
Acceleration $\frac{B-A}{A}$	29	1.4	

Influence of the concentration of serum. Using a very weak invertase (0.1 % of the commercial preparation), the effect of varying concentrations of serum was determined (Table III).

Table III.

% serum present	% inversion of sucrose in $2\frac{1}{2}$ hrs.
10	40
5	32
2.5	20
1.2	6
0	0

Fig. 2. Influence of serum concentration on its acceleration of yeast invertase (serum up to 50 %).

In this experiment invertase = 0.05 %, sucrose = 5 %, $p_H = 4$.

For low concentrations of serum the relationship of serum concentration to invertase activity seems to be linear.

In Fig. 2 is shown the influence of serum concentration up to 50 %. According to this curve the optimal concentration for serum acceleration is about 20 %.

Effect of heat and of chloroform on the invertase-accelerator. Four tubes were incubated as follows:

	Tube 1	Tube 2	Tube 3	Tube 4
	cc.	cc.	cc.	cc.
Invertase	0.1	0.1	0.1	0.1
Sucrose 5 %	0.1	0.1	0.1	0.1
Buffer p_H 4	0.8	0.7	0.7	0.7
Serum	—	0.1	—	0.1
Serum, heated at 70° for $\frac{1}{2}$ hr.	—	—	0.1	—

To tube 4 was added a layer of chloroform, and the tube contents were shaken up before incubation.

After 2 $\frac{1}{2}$ hours the amount of inversion was determined in the usual manner (Table IV).

Table IV.

No. of tube	Contents	% inversion in 2 $\frac{1}{2}$ hrs.
1	Invertase only	0
2	Invertase plus unheated serum	40
3	Invertase plus heated serum	22
4	Invertase plus serum plus $CHCl_3$	50

The above experiments indicate that heating to 70° for $\frac{1}{2}$ hour impairs, but does not destroy, the invertase-accelerator of serum. Chloroform appears to enhance the action, but this result was not confirmed.

Biological variations. The sera of cow, sheep, rat and man were examined for invertase-acceleration, and in all cases this phenomenon was obtained, though in varying degrees.

In the following experiment the invertase-accelerators in the sera of man, sheep, and guinea-pig were compared (Table V). In each case a weak invertase (0.1 % of the commercial preparation) was incubated with 10 % of the serum, and the inversion determined after 21 hours.

Table V.

Source of serum	% inversion
(Serum absent)	37
Man	44
Guinea-pig	82
Sheep	100

The effect of p_H . In the following series of experiments the invertase used was prepared from yeast. 50 g. were ground up with a little distilled water, and the emulsion diluted with water to 300 cc. The mixture was shaken up with 3 g. $CaCO_3$ and incubated for three days at 37°, a layer of toluene being added to prevent bacterial growth. The mixture was then crudely filtered through filter paper, and then further filtered through a Seitz bacterial filter. The

resulting filtrate was a perfectly clear yellow liquid and contained a very active invertase. Clouding and precipitation gradually developed, however, so that Seitz filtration was necessary every two weeks; the activity of the invertase, however, remained unimpaired by these changes.

The effect of p_H was determined for sheep-serum and for guinea-pig-serum only. The range chosen was from 3 to 8. In each case 12 tubes were incubated as follows:

Contents	Tube											
	1	2	3	4	5	6	7	8	9	10	11	12
Invertase 10 %	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Sucrose 10 %	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Buffer	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7
Distilled water	0.2	0	0.2	0	0.2	0	0.2	0	0.2	0	0.2	0
Serum	0	0.2	0	0.2	0	0.2	0	0.2	0	0.2	0	0.2
p_H of buffer added	3	3	4	4	5	5	6	6	7	7	8	8

In addition, two control tubes were put up—one of serum with sucrose, and one with serum alone. The latter gave the glucose content of serum (which was subtracted from the total reducing sugar found in the reaction tubes), and the former acted as a control for serum-invertase, which was never found.

Sheep-serum. Fig. 3 illustrates the results obtained with sheep-serum after

Fig. 3. Influence of p_H on invertase-accelerator of sheep serum.
In this experiment invertase = 0.5 %, sucrose = 5 %.

18 hours' incubation. From each tube 0.1 cc. was removed and added to 1.4 cc. distilled water. 0.1 cc. of the mixture was analysed for invert sugar by Hagedorn and Jensen's method; by calculation the maximum invert sugar obtainable from this amount is 0.35 mg.

Guinea-pig-serum. 12 tubes were incubated as in the case of sheep-serum, a weaker invertase (0.2 % instead of 0.5 %) being used. These results are illustrated in Fig. 4.

Fig. 5 shows the influence of guinea-pig-serum on a much weaker invertase and a more dilute substrate (0.05 % invertase and 0.5 % sucrose).

It will be noticed that (a) at p_H 5 serum has no influence on invertase in the case of both sheep and guinea-pig: (b) sheep-serum accelerates invertase

only in the neighbourhood of p_H 3—there is a definite inhibition of invertase at p_H 5: (c) guinea-pig-serum possesses a definite zone of invertase-inhibition

Fig. 4. Influence of p_H on invertase-accelerator of guinea-pig-serum.
In this experiment invertase=0.2 %, sucrose=5 %.

in the neighbourhood of p_H 6, this zone expanding with increase in the concentration of invertase used.

Fig. 5. Influence of p_H on invertase-acceleration of guinea-pig-serum.
In this experiment invertase=0.05 %, sucrose=0.5 %.

SUMMARY.

1. Serum does not normally contain invertase.
2. Intraperitoneal injections of sucrose do not give rise to serum-invertase.
3. It is possible to produce invertase in serum by intraperitoneal injections of invertase.
4. Serum possesses the power of accelerating yeast-invertase. This invertase-accelerator possesses the following properties.
 - (1) It acts only on dilute invertase.
 - (2) It is not destroyed by heating to 70° for 30 minutes, a treatment destructive to invertase.
 - (3) It is not diminished by shaking the serum with chloroform.
 - (4) The extent of acceleration varies with the concentration of serum: the relationship is linear for weak concentrations of serum: acceleration is at a maximum at 20 %, and diminishes at higher concentrations of serum.

(5) The effect of p_H is very striking. In both sheep and guinea-pig there is no influence on invertase action at p_H 5: in sheep-serum, using less dilute invertase, acceleration is obtained only at p_H 3, while at p_H 4 and above p_H 5 there is invertase-inhibition: in guinea-pig-serum invertase-inhibition occurs only at p_H 6, while acceleration is obtained at p_H 3 to 5 and 7 to 8, when very weak invertase is used: increasing the concentration of invertase widens the p_H band of inhibition.

5. Invertase-accelerator is present in the sera of man, sheep, cow, guinea-pig, and rat.

6. Lipase-accelerator is the only other enzyme-accelerator so far demonstrated in serum. An amylase-accelerator has been suggested, but the evidence is unsatisfactory.

Acknowledgments are due to Prof. D. B. Blacklock (formerly Director of the Sir Alfred Lewis Jones Laboratory, Freetown), and to Dr McCartney, Director of the Pathological and Research Laboratories of the Metropolitan Asylums Board, London, for facilities granted, and for permission to publish details of experiments carried out in their respective laboratories.

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