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A new method for testing the interaction of ferments and anti-ferments.

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Within the past two years, the antitryptic activity of human serum has been the subject of a considerable number of studies, and it has apparently become established that this property of the serum varies within wide limits in certain conditions of disease, being characteristically increased in all cachexias, notably so in cancer. Two methods have been employed in this study, which are exactly the same in principle, in that they are based on the use of a series of mixtures of trypsin in solution, and of the serum, the end-point in the one method being denoted by the smallest quantity of serum which totally inhibits the digestive action of the trypsin, on a plate of coagulated beef-serum, while, in the other, it is the lowest proportion of trypsin which completely digests the medium, namely, a solution of casein. It is evident that by either of these methods it is possible to determine only an approximate value of serum, lying between the two dilutions, just above and just below the so-called end-point. Moreover, the end-point itself is in neither method sharply defined. If, to these uncertainties, is added the fact that the media of digestion are subject to certain uncontrollable variations, such as the inherent differences in sera,— it will be

manifest that the methods are materially lacking in exactness. Consequently, the results have been divergent.

The viscosimeter * appears to offer a method which avoids these difficulties, and gives constant and reliable quantitive results. The viscosimeter is a capillary tube, as designed by Ostwald, on which there are two markings; the time is measured which is required by a solution to flow from the upper of these markings to the lower. Constant conditions of temperature are afforded by the use of a constant water bath with glass walls to permit the readings. A constant medium may be secured by making up a solution of the medium so as to run through the viscosimeter always in a definite space of time. For the purpose of testing tryptic digestion we have used solutions of gelatin. The delicacy of the method may be indicated by the following series of figures for variations of one tenth of one per cent. in the strength of the solution:

Normal salt solution,	70 seconds.
$\frac{1}{10}$ per cent. gelatin in above,	71 "
$\frac{2}{10}$ per cent. " " "	$72\frac{3}{5}$ "
$\frac{3}{10}$ per cent. " " "	$74\frac{3}{5}$ "
$\frac{4}{10}$ per cent. " " "	$75\frac{1}{5}$ "
$\frac{5}{10}$ per cent. " " "	$78\frac{3}{5}$ "
$\frac{6}{10}$ per cent. " " "	$80\frac{2}{5}$ "
$\frac{7}{10}$ per cent. " " "	$82\frac{2}{5}$ "
$\frac{8}{10}$ per cent. " " "	$83\frac{4}{5}$ "
$\frac{9}{10}$ per cent. " " "	$86\frac{4}{5}$ "
1 per cent. " " "	$89\frac{1}{5}$ "

It is evident that the greater the degree of digestion of the gelatin, the lower will be its viscosity and the lower the corresponding figures.

The accuracy with which the digestive action of varying amounts of the ferment upon gelatin may be determined, is indicated by the following series of figures, in which the differences are given for one hundredth of one per cent. of the trypsin, digestion having been interrupted at the end of one hour:

0.5	per cent. trypsin:	2	minutes,	51	seconds.
0.49	per cent. "	2	"	53	"
0.48	per cent. "	2	"	56	"

* The viscosimeter was first used in digestion experiments by Spriggs (*Zeit. f. physiol. Chemie*, 1902, XXXV., p. 480). Owing to the fact that he used a beef extract as his medium, the results were inconstant, and the method failed of any further application.

0.47 per cent.	"	2	"	58	"
0.46 per cent.	"	2	"	58	"
0.45 per cent.	"	3	"		
0.44 per cent.	"	3	"		
0.43 per cent.	"	3	"	02	"
0.42 per cent.	"	3	"	02	"
0.41 per cent.	"	3	"	04	"
0.4 per cent.	"	3	"	04	"

The accuracy with which the antitryptic value of serum can be determined, is illustrated by the following series of tests:

1 c.c. 1	per cent.	serum	+ 1 c.c. 0.2	per cent.	trypsin,	1 min.	57 sec.	
1 c.c. 2	per cent.	"	+ 1 c.c. 0.4	per cent.	"	1 "	58 "	
1 c.c. 1.5	per cent.	"	+ 1 c.c. 0.2	per cent.	"	2 min.		
1 c.c. 3	per cent.	"	+ 1 c.c. 0.4	per cent.	"	2 "		
1 c.c. 2	per cent.	"	+ 1 c.c. 0.2	per cent.	"	2 "	2 "	
1 c.c. 4	per cent.	"	+ 1 c.c. 0.4	per cent.	"	2 "	2 "	
1 c.c. 2.5	per cent.	"	+ 1 c.c. 0.2	per cent.	"	2 "	4 "	
1 c.c. 5	per cent.	"	+ 1 c.c. 0.4	per cent.	"	2 "	5 "	

Thus, it will be seen that the method permits of a very accurate estimation of the action of ferment at any given moment, and of the restraining action of anti-ferment, which can be progressively followed over considerable periods of time. It necessitates the use of only one dilution, instead of a series, as in previous methods. It determines the end-point exactly, instead of approximately. It determines variations in the media by means of the use of controls in each experiment, which is not possible by the methods now in use.

In testing human sera with a gelatin of an original viscosity time of four minutes, a trypsin of 0.5 per cent. strength and sera of 2 per cent. dilution, the range of inhibition covers approximately 60 seconds, so that the values of sera may be far more sharply differentiated than is possible with a series of five dilutions, as in the methods hitherto used.

We shall not give the details of the tests hitherto made, which embrace about two hundred sera of various conditions of disease. It is, however, worthy of emphasis, we believe, that the so-called anti-tryptic action of human sera manifests altogether different relative values when tested against the proteolytic ferment present in a glycerin extract of cancers as compared with the commercial trypsin derived from animals.