CCVI. THE IMMUNOLOGICAL REACTION TO THE ENZYME PAPAIN.

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(Received August 9th, 1932.)

REPORTS have been highly contradictory with respect to the possible development of increased anti-enzymic activity in the serum of a subject which has received repeated injections of a given enzyme preparation. Wells [1925], in reviewing the subject, considers the general thesis very much open to doubt. Probably the most definite evidence of a positive nature is the work by Kirk and Sumner [1931; 1932] with crystallised urease preparations. This particular enzyme is much more stable in the presence of serum than most enzymes and such a property would seem to be a requisite for the production of an anti-enzyme. Northrop [1930], for instance, failing to obtain anti-enzymic effects with a crystallised pepsin preparation, attributed the failure to the recognised instability of pepsin in blood-serum. The vegetable enzyme, papain, by virtue of its relative stability in serum [Walton, 1931, 1, 2] is of special interest in this regard. In the previous studies [Ehrenreich, 1900; Stenitzer, 1908; Achalme, 1901; Pozerski, 1909] concerning the possible development of an antipapain effect proteolytic assay methods have been used which were evidently of slight sensitiveness. For instance, the presence of fluctuating amounts of serum-proteases in the same animal was not recognised in such studies. Ehrenreich [1900] and Achalme [1901] were unable to obtain antipapain effects with rabbits and guinea-pigs, while Stenitzer [1908] reported an anti-enzymic effect in one case with a goat. On the basis of these considerations, the problem was reinvestigated, as reported here, using a method of proteolytic assay which is unusually accurate at relatively high dilutions. The sensitiveness of this particular method was such that the development of a relatively slight antipapain effect should have manifested itself in the recorded measurements.

PROCEDURE.

Two healthy dogs of 8 and 10 kg. weight were each subjected to 20 intravenous and 4 intraperitoneal injections of a papain preparation during a period of 97 days. Venous blood was withdrawn at intervals and the serum used in an assay of its effect on the proteolytic activity of a solution of the papain. Samples were always taken before any injections given the same day. Intravenous

injections were borne without apparent immediate effects, while intraperitoneal injections caused considerable discomfort for a few minutes. During the course of the injections the animals lost weight and developed hemorrhagic skin manifestations, but maintained voracious appetites. On discontinuance of the injections they returned to normal.

Sterile papain for injection was obtained by pressure filtration of glycerol-water solutions through Berkefeld candles and preservation of the filtrates in sealed ampoules. Fresh filtrates, prepared about every 3 weeks, were sufficiently stable, as judged by measurements in this work and by a previous standardisation study of the same procedure [Walton, 1931, 1, 2; 1932]. The stock preparations of Parke Davis papain, whose activity has been defined in this same study, were used throughout. Injections of 1 cc. of filtrate (activity equal to about 40 mg. dry powder) are indicated in Fig. 1 by a single arrow and injections of 2 and 3 cc. by double and triple arrows, respectively.

Fig. 1. Ordinates: percentage original activity of a 1-1000 papain solution after being mixed with serum (10 %).

- ↓ Injection of a papain solution equal in activity to 40 mg. dry powder.
- Observations on normal dogs.

The determinations of proteolytic activity were carried out essentially according to the photographic film method as first described by Gates [1930] and by Gilman and Cowgill [1930]. The technique previously detailed by one of us [Walton, 1931, 1, 2; 1932] was modified in that no fixed temperature was used, but rather any constant temperature in the neighbourhood of 30° which would carry the standard digestion solutions (1-1000 papain) to the end-point (50 % digestion of the gelatin film) in about 20 minutes. Standards and unknowns were always run together at the same temperature. The series of digestion mixtures were sufficient in number to ensure an accuracy of $\pm 5\%$. Papain at a 1-1000 concentration in a solution of 1 % sodium citrate was compared in its digestive activity with solutions of papain at similar concentrations but containing 10 % serum. If the serum was completely inert in this system, its 10 % mixture with a standard solution would result in a solution exhibiting 90 % of the activity of the standard solution. Observations with normal serum demonstrate the frequent presence of proteases in sufficient strength to manifest activities as high as 115 % of the standard in mixtures of this sort.

Possible serum changes due to varying intervals between time of sample taking and time of assay were shown to be without significance. Samples of

the two sera assayed at 1½ and 5 hour intervals after withdrawal showed no variation outside the accepted experimental error. These and all samples used in the study were kept on ice during this interval. A more drastic test of this point was made by heating the two sera for 1 hour at 60° before mixing with papain. Whereas the mixtures of papain with unheated sera exhibited activities of 100 % and 110 %, the mixtures with the heated sera exhibited activities of 85 % and 100 %, respectively.

Varying intervals between the time at which the serum was mixed with papain and the time of assaying the mixture were likewise shown to be without significance. Samples of the two sera mixed with papain and assayed immediately manifested substantially the same effect when assayed after the mixture had stood for 1 hour at 25°.

One comparison of the two sera taken before and after feeding showed a decrease in the proteolytic activities of the mixtures after feeding. Sera obtained before feeding exhibited activities of 85 % and 110 % in the usual mixtures with papain, while those obtained after feeding exhibited activities of 77 % and 95 %, respectively. During the course of the study, samples were taken at a regular period about 4 hours after feeding, and, as these figures indicate, some such routine procedure is essential.

RESULTS.

Results are illustrated in Fig. 1. The normal fluctuations of serum-proteases are evidently very appreciable and any study of this type must account for such, either by subtracting the protease effect of the serum at each determination or by obtaining a sufficient number of normal determinations to establish an average. It is apparent from the general direction of the curves that the development of an antipapain effect, if present at all, is very transitory and of slight magnitude. The occasional drop in the curves may as well be due to a disturbed physiology as to an immunological reaction.

A violent reaction which occurred in both dogs on the fourth injection was of an anaphylactic nature in every observable characteristic except for the unusually brief incubation time. No further reactions of this sort occurred, although some of the subsequent injections were 3 times the dosage of that which provoked the reaction.

The sera gave precipitin reactions with filtered solutions of the papain. However, observations of this sort are complicated by the fact that normal sera gave apparent precipitin reactions with papain solutions.

Towards the latter part of the study, serum-papain mixtures of the proportions previously described were assayed before and after centrifuging off the precipitate obtained by their mixture. After removing the precipitates, which were of very appreciable bulk, the supernatant fluids exhibited substantially the same proteolytic activity as the uncentrifuged portions of the same mixture. Apparently, the proteolytic component of the papain preparations is not subject to such precipitation.

SUMMARY.

A series of observations has demonstrated the absence of any consistent tendency toward development of an anti-enzymic agent on continued injection of papain. Factors influencing the determinations of such possible effects are evaluated.

REFERENCES.

- Achalme (1901). *Ann. Inst. Pasteur*, **15**, 737.
Ehrenreich (1900). Inaugural Dissertation, Würzburg.
Gates (1930). *Science*, **72**, 147.
Gilman and Cowgill (1930). *J. Biol. Chem.* **88**, 743.
Kirk and Sumner (1931). *J. Biol. Chem.* **94**, 21.
—— (1932). *Proc. Soc. Exp. Biol. Med.* **29**, 712.
Northrup (1930). *J. Gen. Physiol.* **13**, 739.
Pozerski (1909). *Ann. Inst. Pasteur*, **23**, 205, 321.
Stenitzer (1908). *Biochem. Z.* **9**, 383.
Walton (1931, 1). *J. Pharm. Exp. Ther.* **43**, 487.
—— (1931, 2). *Proc. Soc. Exp. Biol. Med.* **28**, 922.
—— (1932). *J. Lab. Clin. Med.* **17**, 459.
Wells (1925). *Chemical pathology*, 5th ed.