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The Effect of Adjuvant Disease on Serum Amylase in the Rat (38603)

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A useful model of inflammation which approximates rheumatoid arthritis in man is adjuvant induced polyarthritis in the rat. This debilitating systemic disease, first described by Stoerck (1) and by Pearson (2) is provoked by a single injection of a mixture composed of heat killed mycobacteria in liquid paraffin. In addition to the pathologic changes of the skeletal and connective tissues, it is well documented that rats with adjuvant disease also suffer aberrant liver function. Included are decreased glycogen storage (3), derangement in various enzymic capacities to metabolize drugs (4, 5), decreased albumin–mercaptalbumin synthesis (6–8), and enhanced synthesis of alpha-macroglobulins and fibrinogen (9).

Since it is generally held that the liver is an important regulatory organ for serum amylase, we thought it would be of interest to study the effect of adjuvant disease on this enzyme.

Materials and Methods. Male Charles River, Lewis strain rats weighing 150 g at the start were used. Heat killed mycobacterial cells (Difco) in light mineral oil were injected into the subplantar area of the hindpaw. At appropriate times thereafter cardiac blood was withdrawn, allowed to clot, and the serum harvested. In some instances urine samples were also taken. Amylase activity was assayed immediately. Each sample was diluted 50-fold in sodium phosphate buffer, 0.02 *M*, pH 7.0, with 0.005 *M* sodium chloride. To a 0.2 ml aliquot of each diluted sample was added 1.0 ml of dyed soluble starch substrate (DyAmyl-L Warner-Lambert Co.). After 10 min at 37° unhydrolyzed starch was precipitated with 5 ml of DyAmyl precipitant solution. Following centrifugation the supernatant was decanted and its optical density at 540 nm minus the optical density of an identical sample precipitated at zero time was taken as a measure of amylase activity. The values given in the tables are average optical densities ±SE obtained using DyAmyl-L reagents. A calibration curve relating optical density to amylase units was constructed using samples of reference sera (Versatol E and Versatol E-N, Warner-Lambert Co.). Under conditions of the test an undiluted 0.2 ml sample yielding an optical density of 0.250 represents approximately 110 DyAmyl units/100 ml of serum.

It should be mentioned that alternative amylase methods, e.g. iodine color (11), maltose appearance (10), glucose appearance (12) confirm the findings given below.

Results. It was found that sera obtained from rats injected with mycobacteria exhibited a reduced amylase activity. Data given in Table I indicate that sera from arthritic rats contained about half the activity of that found in uninjected rats. The amount of mycobacterial substance required to bring about this change was greater than 100 μg.

Shown in Table II are serum amylase activities found at various time intervals after injection of *M. butyricum*. The reduced amylase activity was not apparent until the second week and persisted until day 21 and in some cases until day 28. Also shown in the table is the lack of effect of the 50 μg dose of *M. butyricum* over the entire treatment period. Occasionally a reduction was seen with 1000 μg doses as early as day 2.

An analagous decline in the amylase activity of urine was found and its time course is given in Table III. Seven days after a 500 μg injection no change in either serum or urine amylase was apparent. However, by day 14 both activities had declined and both remained depressed through day 21.

Heated serum (50° for 3 min) from arthritic rats did not inhibit known amylase containing serum. Attempts to activate sera from arthritic animals with chloride or calcium ions were not successful.

Discussion. Many investigators have ob-

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TABLE I. SERUM AMYLASE ACTIVITY 25 DAYS AFTER INJECTION OF *M. BUTYRICUM*.

Treatment	Dose (μg)	Number of rats	Amylase optical density (540 nm) ± SE
None	—	41	0.41 ± 0.02
M. butyricum	100	17	0.40 ± 0.02
M. butyricum	250	29	0.23 ± 0.02[a]
M. butyricum	1000	19	0.19 ± 0.01[a]

[a] Significantly different from control $P < 0.001$.

TABLE II. SERUM AMYLASE ACTIVITY AT VARIOUS TIME INTERVALS AFTER INJECTION OF *M. BUTYRICUM*.

Treatment	Dose (μg)	Day	Amylase optical density (540 nm) ± SE
None	—	2	0.45 ± 0.01
M. butyricum	50	2	0.40 ± 0.01
M. butyricum	500	2	0.48 ± 0.02
None	—	7	0.49 ± 0.01
M. butyricum	50	7	0.49 ± 0.04
M. butyricum	500	7	0.55 ± 0.05
None	—	14	0.46 ± 0.02
M. butyricum	50	14	0.47 ± 0.04
M. butyricum	500	14	0.27 ± 0.01[a]
None	—	21	0.33 ± 0.01
M. butyricum	50	21	0.30 ± 0.01
M. butyricum	500	21	0.17 ± 0.01[a]

[a] Significantly different from control $P < 0.005$. Each group contained 10 rats.

served hepatic injury during acute or chronic episodes of stress and inflammation. Somogyi (13) showed a decrease in serum amylase in human cases of liver injury commenting that "lowered blood diastase is found in cases in which some form or other of liver damage and consequent impairment of liver function is present." Later Gray *et al.* (14) concluded that prolonged debilitating illness was accompanied by hepatic damage. Most recently Bhutta and Rahman (15) surveyed human subjects with diagnosed liver dysfunction and found amylase values well below the normal value.

An accelerated red blood cell sedimentation rate has long been recognized as a reliable index of inflammation. The etiology of this phenomenon has been related to an elevated plasma level of macromolecules

TABLE III. URINE AND SERUM AMYLASE ACTIVITIES AFTER INJECTION OF 500 μg OF *M. BUTYRICUM*.

Treatment	Day	Amylase	
		Serum	Urine
None	7	0.39 ± 0.02	0.33 ± 0.03
M. butyricum	7	0.32 ± 0.02	0.26 ± 0.06
None	14	0.33 ± 0.03	0.36 ± 0.01
M. butyricum	14	0.06 ± 0.01[a]	0.18 ± 0.03[a]
None	21	0.41 ± 0.02	0.39 ± 0.02
M. butyricum	21	0.23 ± 0.02[a]	0.11 ± 0.02[a]

[a] Values differ significantly from control $P < 0.001$. Each group contained 10 rats.

(fibrinogen) synthesized by a responsive liver (16). Elevated plasma fibrinogen, a nonspecific stress response may be induced by microbial agents or their products (17, 18), by polymers like dextran or carageenin (19, 20) or by frank irritants such as formalin (21). Not only is the liver metabolism of fibrinogen altered but also changes are seen in numerous microsomal and soluble enzymes (3, 22, 23).

The decrease in amylase reported here appears paradoxical in the light of work by Zwadyk and Snyder (22) who found an enhanced serum amylase in endotoxin treated mice.

The observed reduction in amylase may represent a number of factors. Undoubtedly the often reported toxicity of the mycobacterial substance (24–26) must play a role. Perhaps the undetected presence of inhibitors or depletion of activators regulates the amount of amylase in the serum. Alternatively, a defect in the transport of the enzyme from the liver to the circulation might be involved. Finally, it must be considered that synthesis of the enzyme by the liver is curtailed and causes the observed deficit.

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