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Research Article

PEPTIDASE ACTIVITY IN HUMAN SERUM FOLLOWING BONE FRACTURE

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In a previous communication (Barber *et al.* [1]) it has been shown that there exists in human serum a cobalt-activatable enzyme which splits l-leucylglycylglycine (LGG). The rate of hydrolysis in normal adult individuals is between 5.5 and 8.5% per hour (in the previous communication the rate of hydrolysis of LGG by serum of normal adult controls was stated to be 6.1 to 7.8% per hour. This value had to be revised on the basis of more subjects investigated since then).

This investigation was originally planned for the clinical study of tissue reactions of the ageing organism. The present communication is not immediately related to this problem but seems to have considerable pathophysiological significance: following fractures there was found to be an abnormal rise in the activity of the LGG-splitting enzyme in human serum.

Ever since the studies by Cuthbertson (2) and Browne (3) and their co-workers, numerous clinical and experimental investigations have been made on the metabolic aspects of injury and repair. There have been many attempts to explain these observations on the basis of one hypothesis but so far no single mechanism has been discovered which could account for the irregularities of protein metabolism accompanying injury and repair. Therefore, every new observation is significant in spite of the fact that its functional meaning may not be clear on the basis of our present-day concepts.

METHODS AND MATERIAL

Blood was obtained by venous puncture from nine patients who had suffered fractures of varying degree. Serum was prepared immediately by centrifugation and stored for not more than 24 hours in a refrigerator. The tri-peptide l-leucylglycylglycine was used as substrate for the determinations which were carried out in triplicate in 2.0 cc. volumetric flasks. The reaction mixture, made up of 0.2 cc. serum per cc., 0.001 M cobalt sulphate, 0.05 M LGG, was kept near pH 7.8 with 0.01 M phosphate buffer and made up to volume with redistilled CO_2-free water. To each tube, 0.01 ml. toluene was added as preservative. Flasks containing serum and substrate controls were prepared, and a zero-time titration was carried out on 0.2 ml. aliquots of the test solutions, according to the microtechnique of Grassmann and Heyde (4). The preparations were then incubated in a water-bath at 39° for six hours, during which time three titration readings were taken. Corrections were made for the controls and the results plotted on a graph. The rate of hydrolysis was calculated from the slope of the zero-order plot of per cent hydrolysis versus time (Barber *et al.* [1]).

In one case (OT), in addition to the peptidase activity, the white blood count, differential, and sedimentation rate were investigated.

RESULTS

The results are demonstrated in Tables I and II and in Figure 1. We see that out of nine cases of fracture the value of hydrolysis was abnormally high in eight.

TABLE I

Serum peptidase activity following fracture

Name	Sex	Age	Nature of injury	Time after injury	Hydrolysis of LGG % per hr
BE	M	40	Crushing injury to left hand	1 day	15
			Loss of tip of index finger	7 days	10.4
BR	M	58	Compound fracture of left fibula	4 days	13
DI	M	62	Right leg fracture	1 day	12.6
KL	F	48	Fracture lower end of radius	3 hours	6.7
LE	F	29	Fractured pelvis	1 day	14
WE	F	35	Lumbar fracture	10 hours	14.6
			Fractured arm	6 days	8.4

The one exception was a case of fracture of the lower end of the radius. It is noteworthy that in this case the blood was obtained sooner after the injury (three hours) than in any other. In another case (lumbar and arm fracture combined) the time interval between injury and blood examination was 10 hours; in this case the rate was 14.6% which belongs to the highest range

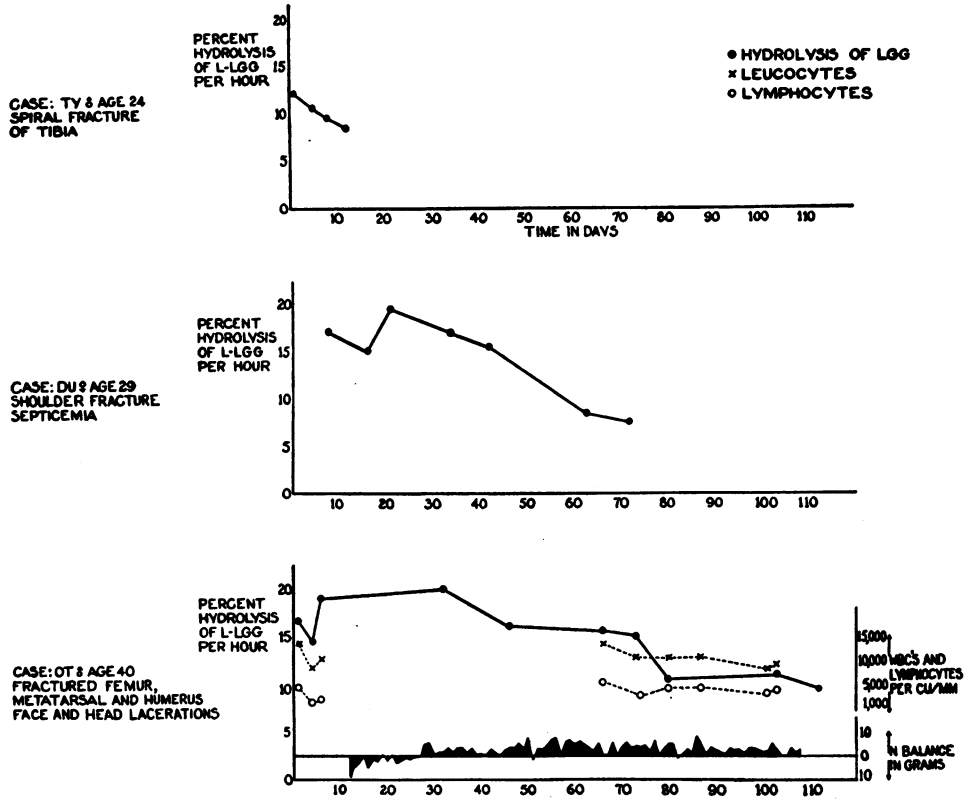

FIG. 1. GRAPHIC PRESENTATION OF THE THREE CASES WHICH WERE STUDIED LONGITUDINALLY

The solid black area in the lowest graph (Case OT) represents nitrogen balance in grams (for further explanation see text).

of the present series. In the remaining seven cases the time interval for the first blood examination ranged from 24 hours to eight days.

In the three cases in which longitudinal studies were made during the period of convalescence we see a tendency to a gradual decline from a high towards a normal value. In one case (TY) this comes close to a linear relationship between activity of the enzyme and duration of the healing process. In the other cases the return to normal is more irregular but it is noteworthy that the convalescence in these instances was complicated. In case DU septicaemia occurred; in case OT there were multiple fractures with widespread lacerations. The latter case was not regarded as completely healed at the time of completion of this paper. It is known that every fracture causes an increase in the erythrocyte sedimentation rate. Hauck (5) states that a delay in the return to normal of this factor would reveal disturbances of consolidation of the injured bone. In Table II it may be observed that in case OT the sedimentation rate is still elevated when the serum pepti-

TABLE II

The relationship of erythrocyte sedimentation rate to serum peptidase activity in Case OT **

Day after fracture	Sed. rate	Corr. sed. rate	Compact cells	% Hydrolysis of LGG per hr.
	mm./hr.	*mm./hr.*	*mm.*	
1	31	11	33	16.2
4	68	24	25	14.5
6	69	20	25	18.8
32	—	—	—	20
47	42	36	44	16
66	40	34	45	15.5
73	40	28	40	15
80	43	28	39	10.2
103	35	43	28	11.5
110	37	32	44	9

* This patient, male, aged 40, suffered compound fractures of the left femur, left metatarsal and fracture of left humerus, with numerous head and face lacerations.

dase activity has practically reached the normal value.

DISCUSSION

We see that in eight out of nine cases of fracture the activity of the LGG peptidase of the serum was significantly increased. The one exception was a case of a relatively minor fracture in which the blood was taken sooner after the accident than in any of the remaining cases. On the other hand, the patient whose peptidase activity was highest (20% on the 32nd day) had suffered the most widespread and severe injuries. This would, at first glance, suggest a correlation between severity of injury and degree of peptidase activity; however, the number of cases is too limited to allow such a conclusion.

What is the possible mechanism of this phenomenon? Fruton (6) discussed the widespread distribution of peptidases capable of splitting LGG, in intestinal mucosa, in lymph and muscle, and in leucocytes and lymphocytes, suggesting that they may have a common origin in the leucocytes and lymphocytes invading the tissue. Holman, White and Fruton (7) observed an increase in the manganese-activatable LGG serum peptidase activity following the injection of adrenocorticotropic hormone into mice and rabbits, and considered this to be associated with the resultant turnover of lymphoid tissue. If this is so, then our observed increases in peptidase activity during the healing of fractures might be directly correlated with the leucocytic reaction at the site of the fracture. Moritz (8) states that the reactive inflammation with exudation begins "soon after the injury has been sustained and reaches its maximum between ten days and two weeks." From experiments carried out in our laboratory, the injection of adrenocorticotropic hormone into normal control subjects is not followed by any striking increase in the activity of the cobalt-activatable serum peptidase.[1] Moreover, in cases of acute infections there was no parallel between the numbers of circulating lymphocytes and the activity of this enzyme.

The changes in protein metabolism associated with injury and repair have been discovered comparatively late (Cuthbertson [9]). Since then a wealth of observations has been produced. From the careful review by Beattie (10) it is quite obvious that there is no one theory on which the various metabolic phenomena associated with injury and repair could be explained. In only one of our cases (Figure 1, Case OT) was it possible to observe the nitrogen balance [2] together with the peptidase activity; in this case the peptidase activity began to decline with the onset of a positive nitrogen balance. Ingle *et al.* (11) demonstrated that adrenalectomized rats did not show the characteristic negative phase of the N-balance following fracture. From this it would seem that the presence of an adrenal hormone is essential for this reaction. However, as noted above, in normal human subjects we failed to obtain any striking increase in serum peptidase activity following injection of adrenocorticotropic hormone. Thus we see that fractures are associated with a rise and return to normal of the peptidase activity of the serum during the course of healing. However, on the basis of present-day knowledge we have no definite theoretical explanation of this phenomenon. Observations like these may well lead to a procedure which would be clinically useful in the evaluation of wound healing.

[1] Observations to be published.

SUMMARY

Eight out of nine cases of fracture showed in their blood serum an increase in the activity of the cobalt-activatable enzyme which hydrolyses LGG. In three cases studied longitudinally the rate of hydrolysis returned to normal during the course of healing. In one of these cases nitrogen balance studies were done simultaneously. In this case the activity of the enzyme began to decline when a positive nitrogen balance was established.

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[2] The authors are indebted to Dr. J. S. L. Browne and Dr. L. G. Johnson of the University Clinic, Royal Victoria Hospital, Montreal, for the data on the nitrogen balance study shown in Figure 1.

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