ance and terminally it contains a decreased amount of collagen with considerable admixture of precollagen. The scar is less dense than that of the control group, but more dense than that of the pyridoxin-deficient group. The vascularity and cellularity are increased.

*Discussion.* Disturbance in the production of collagen is generally considered characteristic of scurvy, but is also known to occur in hypoproteinemia. The results obtained in the present experiments indicate that a similar disturbance is associated with deficiency of biotin, pyridoxin, or riboflavin in rats. However, the retardation of collagen production in these vitamin-deficiencies is less pronounced and less persistent than in scurvy.

The pyridoxin- and riboflavin-deficient groups exhibit, in addition, delayed contraction of the wound as observed grossly, and tardy exudation; the latter resulting in delayed sequestration and production of granulation tissue. The residual increased vascularity and correlative decreased density of the scars of the pyridoxin- and riboflavin-deficient groups are further indications of retardation in wound healing which are logically associated with the terminally decreased collagen and increased precollagen.

The wound of biotin-deficient animals exhibits a somewhat diminished density and decreased collagen content of the scar. There is no retardation of exudation or of granulation tissue production. Terminally the scar is indistinguishable from that of a normal animal.

The role which inanition plays in the retardation of wound healing is difficult to assess. It may or may not be coincidence that the greatest retardation in wound healing occurs in animals where inanition is also maximal—in the riboflavin- and pyridoxin-deficient groups. However, while the retardation in wound healing is greater in pyridoxin-deficient rats than in the riboflavin-deficient animals, inanition is greater in the latter than in the former.

*Summary.* The process of wound healing has been observed in rats exhibiting severe deficiency of biotin, pyridoxin, or riboflavin. Marked impairment of rate and quality of healing was noted in the pyridoxin- and riboflavin-deficient groups. The biotin-deficient group showed only mild delay in healing.

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## Peptidases in Human Serum.*

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(Introduced by J. S. L. Browne.)

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Although the existence of proteolytic enzymes in blood serum and leucocytes has long been recognized, no definitive work has been done in this field until recent years (cf. review of the early literature, Opie[1]).

With the development of the use of synthetic di- and tri-peptides as substrates, a quantitative microtitration technique was devised for the determination of peptidase hydrolysis (Grassmann and Heyde,[2] Abderhalden and Hanson,[3] Maschmann,[4] Fruton,[5]

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1 Opie, E. L., *Physiol. Rev.*, 1922, **2**, 552.

2 Grassmann, W., and Heyde, W., *Z. physiol. Chem.*, 1929, **183**, 32.

3 Abderhalden, E., and Hanson, H., *Fermentforsch.*, 1937, **15**, 382.

4 Maschmann, E., *Biochem. Z.*, 1941, **308**, 359.

5 Fruton, J. S., *J. Biol. Chem.*, 1946, **166**, 721.

TABLE I.
Hydrolysis of LGG, GL, and GG by Normal Human Serum.
(The hydrolysis of LGG is most rapid and considerably activated by cobalt ions.)

| Substrate .05 M | cc human serum per cc test sol. | Metal ion | Hydrolysis *% per hour |
|---|---|---|---|
| LGG | .4 | ——(avg of 5) | 7.3 |
| | .4 | Mg (.01 M) | 5.0 |
| | .4 | Mn (.001) | 5.5 |
| | .4 | Co (.001) | 9.6 |
| GL | .4 | ——(avg of 4) | 2.3 |
| | .4 | Mg (.01 M) | 1.2 |
| | .4 | Mn (.001) | 3.4 |
| | .4 | Co (.001) | 2.5 |
| GG | .4 | —— | 0.0 |
| | .4 | Co (.001 M) | 3.0 |

* % per hour of the hydrolysis expected on the complete splitting of one peptide linkage.

and Holman *et al.*[6]). Except for some preliminary observations on pathological human serum with l-leucylglycylglycine as substrate (Grassmann and Heyde[7]) the method has found no clinical application thus far.

A study was undertaken to investigate proteolytic activity of the human serum in clinical conditions associated with ageing. As a preliminary step, however, it was necessary to study this function in normal control subjects and under various conditions involving leucocytosis and tissue proliferation.

*Method.* Blood was obtained from nonfasting subjects by venous puncture. Serum was prepared by centrifugation.

The tri-peptide l-leucylglycylglycine (LGG) and the di-peptides glycyl-l-leucine (GL) and glycylglycine (GG) were used.† In the activation studies the method described by Maschmann[4] was followed, in which 0.01 M cobalt, 0.01 M manganese or 0.1 M magnesium were added as sulphates. The reaction was carried out in a water bath at 39°C in 2.0 ml volumetric flasks containing 0.05 mM/cc of the substrate. The pH was maintained near pH 7.8 with 0.01 M phosphate or veronal buffer, both of which were found to be equally effective. 0.01 ml of toluene was added to each tube as preservative. In the preliminary experiments on LGG, GG and GL, 0.4 cc of serum per ml of reaction mixture were used. With cobalt as activator, the concentration of serum was reduced to 0.2 cc per ml of test solution in the studies on LGG. The experiments on the 11 normal control subjects and the pathological cases were carried out on LGG with this serum concentration. The rate of proteolytic hydrolysis was followed in triplicate according to the titration method of Grassmann and Heyde.[2] Four readings were taken over a period of 7 hours, following a zero time determination. The results were corrected for serum and substrate controls which were run simultaneously. The rate of activity per hour was calculated from the slope of the zero order plot of the per cent hydrolysis versus time (per cent hydrolysis equals per cent of the hydrolysis expected on the complete splitting of one peptide linkage).

*Subjects.* Eleven members of the staff (5 males and 6 females) ranging in age from 24 to 47 years were studied as normal controls. Investigations were carried out on 10 subjects suffering from various pathological conditions. These included dental extraction, postpartum infections, fractures, aleukemic leukemia, cancer and non-malignant tumor.

*Results.* The results are presented in Tables I and II. In the serum of 11 normal control subjects the rate of hydrolysis ranged from 6.1 to 7.8%/hour with an average of $7.2 \pm 0.78\%$.

[6] Holman, H. R., White, A., and Fruton, J. S., PROC. SOC. EXP. BIOL. AND MED., 1947, **65**, 196.

[7] Grassmann, W., and Heyde, W., *Z. physiol. Chem.*, 1930, **188**, 69.

† These peptides were obtained through the courtesy of Dr. M. S. Dunn at the University of California in Los Angeles.

TABLE II.
Action of Serum Peptidases in Various Abnormal Conditions.*

| Diagnosis | Condition | Age | Sex | Hydrolysis % per hr |
|---|---|---|---|---|
| Vulvar carcinoma, epilepsy | Sed. rate 39 | 39 | F | 9.4 |
| Cervical carcinoma | " " 30 | 47 | F | 5.2 |
| " " | " " 52 | 52 | F | 10.9 |
| Non-malignant tumor | " " 49 | 46 | F | 6.4 |
| Aleukemic leukemia | WBC: 6,500/cu mm<br>Lym.: 4,680/cu mm<br>High sed. rate | 47 | M | 7.4 |
| Postpartum pneumonia | 6th day postpartum | 23 | F | 11.0 |
| | 10th " " | | | 7.6 |
| " infection | Penicillin treatment<br>10th day postpartum | 29 | F | 9.6 |
| Leg fracture | Fever 4th day after fracture | 58 | M | 13.0 |
| " " | Day of fracture | 62 | M | 12.6 |
| Lumbar and arm fracture | Day of fracture | 35 | F | 14.6 |
| | 6th day | | | 8.4 |
| Dental extraction | 1 day after | 26 | F | 5.5 |

* The reaction mixture consisted of 0.05 M LGG, 0.001 M cobalt, 0.01 M veronal or phosphate buffer; 0.2 cc of serum were used per cc of reaction mixture.

The manner of compiling the data is illustrated in Fig. 1.

*Discussion.* The present investigation shows that there exists in human serum a cobalt-activatable LGG-splitting peptidase. The rate of hydrolysis is uniform in healthy adult subjects and there are considerable deviations from this normal range in various pathological conditions. The results obtained in this study (Table I) agree with the data of Grassmann and Heyde[7] on human serum. Fruton[5] calculated from this data a value of 0.035% hydrolysis per minute (K°LGG) for 0.1 ml of serum per ml of test solution. Since we found direct proportionality between serum concentration and rate of reaction,‡ our data yields a corresponding K°LGG of 0.030±0.01.

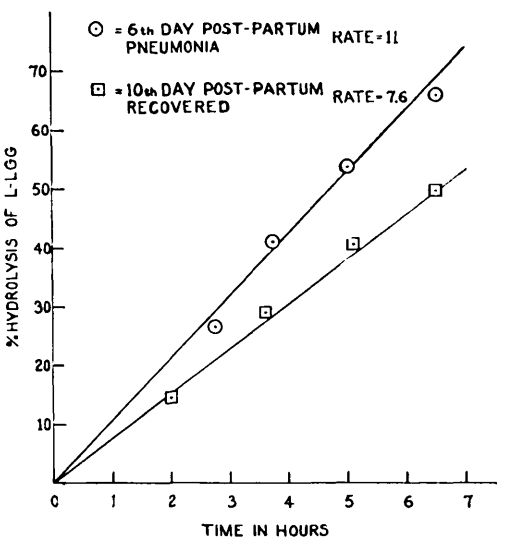


FIG. 1

Hydrolysis of LGG by serum from a case of postpartum pneumonia. It may be seen that the kinetics of the reaction are of a zero order.

From our limited number of cases more detailed conclusions can be drawn only with great reservation. The fact that the rate of hydrolysis was highest in cases of puerperal infection and fractures, and decreased with the progress of healing, suggests further studies along the lines of tissue reaction and repair. This is also indicated by the observations of Zamecnik *et al.*[8] who reported a rise of serum peptidases in animals following burns.

*Summary.* The rate of hydrolysis of l-leucylglycylglycine by the cobalt activatable peptidase in human serum shows little variation in normal adults, but is increased in certain pathological states, particularly in fractures and postpartum infections.

‡ Unpublished experiments.

8 Zamecnik, P. C., Stephenson, M. L., and Cope, O., *J. Biol. Chem.*, 1945, **158**, 139.