

# Effect of Vitamin B<sub>12</sub> on Wound Healing.\* (20156)

CHARLES W. FINDLAY, JR. (Introduced by Edward L. Howes.)

*From the Department of Surgery, College of Physicians and Surgeons, Columbia University and The Surgical Service, Presbyterian Hospital, New York City.*

Vit. B<sub>12</sub> has been found to be an important growth factor in young animals(7,8,11) and several investigators believe the vitamin participates in protein synthesis(2,5,13,14). Numerous studies have emphasized the significance of body proteins in wound healing (1,3,9,10,12). It seemed appropriate, therefore, to investigate the role of B<sub>12</sub> in wound healing of animals with normal and depleted protein stores.

**Method.** Sherman rats weighing about 300 g were used. They were fed Rockland Chow diet prior to the study and under observation they were given one of 3 diets for 3 weeks

before wound infliction and for 6 days post-operatively. A synthetic diet containing 19% protein originally used by Darrow(4) was given to one group of the animals. This was composed of: lactalbumin 18, Crisco 22, dextrin 32, sucrose 25, cod liver oil 1, powdered yeast 2, bone ash 2, NaCl 1, and KH<sub>2</sub>PO<sub>4</sub> 2 g. The second group received a 0.9% low protein diet containing the same components except that lactalbumin was omitted and the proportions of Crisco, dextrin and sucrose were increased to 26, 36 and 29 g respectively. Both these synthetic diets had approximately the same caloric value of about 500 calories

TABLE I. Weight Changes of Animals on Specified Diets and Medication\* before and after Operation.

| Diet†              | Medication                                | No. of rats in group | Wt before diet | Wt after diet for 3 wk | Avg daily intake (g) | % wt change on diet | Wt at death | Avg post-operative daily intake | % wt change since operation |
|--------------------|-------------------------------------------|----------------------|----------------|------------------------|----------------------|---------------------|-------------|---------------------------------|-----------------------------|
| Balanced synthetic | Saline for 6 p‡ days                      | 6                    | 313            | 358                    | 15.6                 | +14.4               | 343         | 15.0                            | -4.2                        |
| "                  | Saline 21 pr and 16 p days                | 6                    | 306            | 344.3                  | 17.7                 | +12.5               | 345         | 16.7                            | + .7                        |
| Rockland chow      | Saline 21 pr and 6 p days                 | 10                   | 307            | 331                    | 18.6                 | + 7.8               | 321         | 16.1                            | -3.0                        |
| Balanced synthetic | Vit. B <sub>12</sub> 6 p days             | 10                   | 301            | 339                    | 15.7                 | +12.3               | 331         | 8.9                             | -2.4                        |
| "                  | Vit. B <sub>12</sub> , 21 pr and 6 p days | 8                    | 279            | 312                    | 13.5                 | +11.9               | 305         | 9.3                             | -1.9                        |
| Rockland chow      | Vit. B <sub>12</sub> 6 p days             | 10                   | 322            |                        |                      |                     | 325         |                                 | + .9                        |
| Low protein        | Saline 6 p days                           | 17                   | 324            | 279                    | 12.6                 | -13.8               | 278         | 11.5                            | - .4                        |
| "                  | Saline 21 pr and 6 p days                 | 11                   | 328            | 275                    | 14.2                 | -16.4               | 270         | 11.6                            | -1.9                        |
| "                  | Vit. B <sub>12</sub> 6 p days             | 14                   | 296            | 252                    | 10.9                 | -14.9               | 241         | 7.2                             | -4.0                        |
| "                  | Vit. B <sub>12</sub> 21 pr and 6 p days   | 8                    | 303            | 258                    | 11.3                 | -14.8               | 250         | 8.9                             | -3.1                        |

\* Rats given vit. B<sub>12</sub> received .5 μ/100 g body wt/day. Daily dosage of isotonic saline to all animals was  $\frac{\text{wt of rat}}{200}$ .

† All specified diets given 3 wk before operation and 6 days post-operatively.

‡ p = post-operation; pr = pre-operation.

\* This work supported in part by a grant from the Medical Research and Development Board, Office of the Surgeon General, Department of the Army.

TABLE II. Data to Determine Effect of Vitamin B<sub>12</sub> on Tensile Strengths of Wounds in Rats Fed Control and Low Protein Diets.

|                                            | Balanced synthetic diet                               |                                              |                                               |                                              | Low protein diet                                      |                                              |                                               |                                                  | Balanced synthetic minus low protein diets       |          | Rockland chow-low protein diets |
|--------------------------------------------|-------------------------------------------------------|----------------------------------------------|-----------------------------------------------|----------------------------------------------|-------------------------------------------------------|----------------------------------------------|-----------------------------------------------|--------------------------------------------------|--------------------------------------------------|----------|---------------------------------|
|                                            | Vit. B <sub>12</sub> or saline, 21 pr* & 6 p days (1) | Vit. B <sub>12</sub> or saline, 6 p days (2) | Diff. between pr & p, & only p admin. (1)-(2) | Vit. B <sub>12</sub> or saline, 6 p days (3) | Vit. B <sub>12</sub> or saline, 21 pr* & 6 p days (4) | Vit. B <sub>12</sub> or saline, 6 p days (5) | Diff. between pr & p, & only p admin. (4)-(5) | Vit. B <sub>12</sub> or saline, 6 p days (2)-(5) | Vit. B <sub>12</sub> or saline, 6 p days (1)-(4) |          |                                 |
| Vit. B <sub>12</sub>                       |                                                       |                                              |                                               |                                              |                                                       |                                              |                                               |                                                  |                                                  |          |                                 |
| Avg tensile strength                       | 125.2                                                 | 133.3                                        | -8.1 (b)                                      | 129.5                                        | 82.0                                                  | 89.4                                         | -7.4 (b)                                      | 43.2 (a)                                         | 43.9 (a)                                         | 40.1 (a) |                                 |
| No. of rats                                | 7                                                     | 7                                            |                                               | 10                                           | 8                                                     | 10                                           |                                               |                                                  |                                                  |          |                                 |
| Infection rate (%)                         | 0                                                     | 0                                            |                                               | 29                                           | 22                                                    | 50                                           |                                               |                                                  |                                                  |          |                                 |
| Saline controls                            |                                                       |                                              |                                               |                                              |                                                       |                                              |                                               |                                                  |                                                  |          |                                 |
| Avg tensile strength                       | 102.0                                                 | 111.9                                        | -9.9 (b)                                      | 108.2                                        | 76.5                                                  | 78.4                                         | -1.9 (b)                                      | 25.5 (a)                                         | 33.5 (a)                                         | 29.8 (a) |                                 |
| No. of rats                                | 6                                                     | 7                                            |                                               | 10                                           | 7                                                     | 15                                           |                                               |                                                  |                                                  |          |                                 |
| Infection rate (%)                         | 0                                                     | 16.6                                         |                                               | 20                                           | 54.5                                                  | 39                                           |                                               |                                                  |                                                  |          |                                 |
| Difference (Vit. B <sub>12</sub> - saline) | 23.2 (a)                                              | 21.4 (a)                                     |                                               | 21.3 (a)                                     | 5.5 (b)                                               | 11.0 (b)                                     |                                               |                                                  |                                                  |          |                                 |

Note: All wounds tested on 6th p day. Designated diets given for 21 pr and 6 p days. Vit. B<sub>12</sub> dosage 0.5 μ/100 g body wt/day. Isotonic saline dosage was wt of rat/200. Student's t-test at .01 levels used to determine significance. (a) designates that the difference is statistically significant. (b) the difference is not statistically significant.

\* pr = pre-operative; p = post-operative.

Note: All wounds tested on 6th p day. Designated diets given for 21 pr and 6 p days. Vit. B<sub>12</sub> dosage 0.5  $\mu$ /100 g body wt/day. Isotonic saline dosage was wt of rat/200. Student's t-test at .01 levels used to determine significance. (a) designates that the difference is statistically significant. (b) the difference is not statistically significant.

\* pr = pre-operative; p = post-operative.

per 100 g of diet. The rest were maintained on the Rockland Chow diet said to contain 25% protein by analysis and with a caloric value of 320 calories per 100 g of diet. The weights of the animals were recorded daily and average daily intakes of food were determined. Percentages of weight change during the pre and postoperative periods were calculated (Table I). The rats on all these diets were given subcutaneously 0.5  $\mu$  of vit. B<sub>12</sub> per 100 g of body weight in saline daily, while the controls received a comparable amount of saline daily. These solutions were given to some animals for the entire experimental period and to others only following operation (Table II). Except as noted later, all rats were killed on the sixth postoperative day. At this time the incidence of wound infection was noted (Table II). The wounds of the non-infected animals were excised, divided into 1 cm strips and tested for tensile strength as previously described(6). From each wound at least 3 segments were tested by a tensionmeter and the results were measured in grams. The strengths of all strips taken from a wound were totaled and divided by the number of segments. This gave the average tensile strength of the wound per cm. Sections were taken for microscopic study.

**Results.** All rats gained weight on the balanced synthetic and Rockland Chow diets and those fed low protein diets for 3 weeks invariably lost weight. Giving vit. B<sub>12</sub> neither increased the food intake nor did it alter the weight changes in any group. This was evident in both the pre and postoperative periods (Table I).

Vit. B<sub>12</sub> given either before and after operation or only postoperatively caused an increase in the tensile strength of sixth day wounds in rats fed the synthetic diet containing 19% protein, and the same effect was noted when B<sub>12</sub> was given postoperatively to Rockland Chow fed animals. No difference was observed in the average tensile strengths for groups given the vitamin both before and after surgery, or only after operation. The tensile strengths of wounds in animals maintained on the low protein diet showed a significant retardation of wound healing when compared to those fed the 19% or 25% protein diets. The administration of B<sub>12</sub> to the low

protein rats caused no significant increase in the strengths of their wounds. For results of statistical analysis of the above differences, see Table II. The low protein rats in general had a high incidence of wound infection by gross examination. (Table II). Vit. B<sub>12</sub> did not check this complication.

Sections of the wounds studied by microscopy showed the usual findings for normal and low protein fed animals. The latter showed diminished fibroplasia, derangement of the normal polarity of the fibroblasts and evidence of tissue edema. The administration of vit. B<sub>12</sub> did not cause microscopic changes in the rats of any group.

Since vit. B<sub>12</sub> when given to rats maintained on balanced diets increased on the average the tensile strength of six-day-old wounds, it seemed important to know when this increased strength was acquired. Accordingly, studies were made on wounds three to eight days old in rats fed balanced synthetic or Rockland Chow diets. Postoperatively they were given parenteral vit. B<sub>12</sub> or saline from the time of operation until death. With the animals on these diets, statistical analysis did not show any difference in the average change of tensile strength from the third to the eighth day when comparing the vitamin groups with the saline controls (Table III). It was determined, however, that the average tensile strength was significantly higher on the third day in the vitamin fed groups. Thus the stronger wounds previously noted at the sixth day in rats fed B<sub>12</sub> could possibly be explained by greater acquisition of tensile strength by the third day. This would indicate that the latent period of healing had been shortened in the rats given B<sub>12</sub>. The continued greater acquisition of strength, on the other hand, was not evident by the eighth day when the vitamin and saline groups were compared. In these two groups, more extensive data will be necessary to detect a significant difference in the change of average tensile strength from the third to the eighth day.

**Summary.** 1) These data show that B<sub>12</sub> increased the tensile strength of wounds during the early phases of healing in rats fed balanced diets containing 19% or 25% protein. This effect was evident at least by the third day in the wounds studied, and it was

TABLE III. Effect of Vit. B<sub>12</sub> on Mean Tensile Strength of Wounds in Rats Fed Balanced Diets from Third to Eighth Day (See Text).

| Post-op days tested       | Rockland chow diet |                      | Balanced synthetic diet |                      |
|---------------------------|--------------------|----------------------|-------------------------|----------------------|
|                           | No. tested         | Tensile strength (g) | No. tested              | Tensile strength (g) |
| Vitamin B <sub>12</sub> * |                    |                      |                         |                      |
| 3                         | 3                  | 54.9                 | 4                       | 51.8                 |
| 4                         | 4                  | 68.7                 | 4                       | 60.7                 |
| 5                         | 6                  | 80.8                 | 5                       | 96.6                 |
| 6                         | 10                 | 129.5                | 7                       | 133.3                |
| 7                         | 7                  | 152.4                | 6                       | 139.8                |
| 8                         | 5                  | 170.0                | 5                       | 173.5                |
| Saline†                   |                    |                      |                         |                      |
| 3                         | 3                  | 39.7                 | 4                       | 42.6                 |
| 4                         | 3                  | 62.0                 | 6                       | 60.3                 |
| 5                         | 5                  | 80.7                 | 5                       | 81.8                 |
| 6                         | 10                 | 108.2                | 7                       | 111.9                |
| 7                         | 5                  | 140.0                | 6                       | 127.4                |
| 8                         | 5                  | 159.7                | 5                       | 179.6                |

\* 0.5  $\mu$ /100 g body wt/day.

† Dosage of isotonic saline was  $\frac{\text{wt of rat}}{200}$ .

Respective diets were given 3 wk before surgery and postoperatively until animal was killed.

most noticeable by the sixth day. 2) The wounds that were studied in the saline control and vitamin treated rats from the third to the eighth day showed no significant difference in their healing rates at the eighth day. 3) The delayed healing rate and increased incidence of wound infection in rats that were protein depleted by diet were not significantly altered by administration of vit. B<sub>12</sub>.

Grateful acknowledgment is made to Phyllis B. Michelsen, Division of Biostatistics of the Faculty of Medicine, Columbia University, for the statistical analyses. The vitamin B<sub>12</sub> used in this experiment was donated by Merck, Inc., Rahway, N. J.

1. Chalkey, H. W., Algire, G. H., and Morris, H. P., *J. Nat. Cancer Inst.*, 1946, v6, 363.
2. Charkey, L. W., Wilgus, H. S., Patton, A. R., and Gassner, F. X., *PROC. SOC. EXP. BIOL. AND MED.*, 1950, v73, 21.
3. Clark, A. H., *Bull. Johns Hopkins Hosp.*, 1919, v30, 117.
4. Darrow, D. C., Schwartz, R., Dannucci, J. F., Coville, F., *J. Clin. Inv.*, 1948, v27, 198.
5. Dempsey, E. W., and Wislocki, G. B., *Physiol. Rev.*, 1946, v26, 1.
6. Findlay, C. W., Jr., and Howes, E. L., *Surgery*, 1950, v28, 970.

7. Hartman, A. M., Dryden, L. P., and Cary, C. A., *Arch. Biochem.*, 1949, v23, 165.
8. ———, *J. Am. Diet. Assn.*, 1949, v25, 934.
9. Kobak, M. W., Benditt, E. P., Wissler, R. W., and Steffee, C. H., *Surg., Gynec., and Obs.*, 1947, v85, 751.
10. Localio, S. A., and Hinton, J. W., *Surg., Gynec., and Obs.*, 1948, v86, 107.
11. Luecke, R. W., McMillen, W. N., Thorp, F., Jr., and Boneice, J. R., *Science*, 1949, v110, 139.
12. Rhoads, J. E., Fliegelman, M. T., and Panzer, L. M., *J.A.M.A.*, 1942, v118, 21.
13. Sahasrabudhe, M. R., and Lakshminarayan, Rao, M. V., *Nature*, 1951, v168, 605.
14. Stern, J. R., Taylor, M. W., and Russel, W. C., *Proc. Soc. Exp. Biol. and Med.*, 1949, v70, 551.

Received February 2, 1953. P.S.E.B.M., 1953, v82.

### Parathyroid Extract and Alkaline Phosphatase Activity.\* (20157)

CHARLES D. KOCHAKIAN, BARBARA A. REED, MARIO BONELLI, AND GIANNI SALA.

From Oklahoma Medical Research Institute,<sup>†</sup> Oklahoma City, and Istituto di Clinica Medica Generale dell'Università di Milano, Italy.

Parathyroid extract has been reported to increase(1) and to have no effect(2) on the alkaline phosphatase activity of the kidneys of rats. This difference now has been resolved by repeating the experiments with each of the separate laboratories performing simul-

taneously both of the previously reported procedures.

**Results.** The results obtained in both laboratories were identical (Table I). The parathyroid extract<sup>‡</sup> produced an increase in phosphorus excretion, but no change in the

TABLE I. Inability of Parathyroid Extract to Change Alkaline Phosphatase Activity of Kidney.\* (5 male rats per group.)

| Treatment                    | B. wt, g  | Urine P, mg (24 hr) | Kidney, g  | Alkaline phosphatase, U/g |          |          |          |
|------------------------------|-----------|---------------------|------------|---------------------------|----------|----------|----------|
|                              |           |                     |            | Homogenate at 5°, 18 hr†  |          |          |          |
|                              |           |                     |            | K-A                       |          | Bodansky |          |
|                              |           |                     |            | pH 9.7                    | 8.6      | 9.7      | 8.6      |
| Bonelli and Sala             |           |                     |            |                           |          |          |          |
| Control                      | 215 ± 10‡ | 5.1 ± 1.7           | 1.64 ± .10 | 56 ± 3.4                  |          | 35 ± 2.2 |          |
| PTH 1 ml                     | 200 ± 6   | 9.6 ± 1.5           | 1.52 ± .05 | 54 ± 6.4                  |          | 32 ± 1.7 |          |
| Kochakian and Reed           |           |                     |            |                           |          |          |          |
| Control                      | 209 ± 6   | 7.7 ± 1.7           | 1.52 ± .07 | 111 ± 5.0                 | 46 ± 4.9 | 36 ± 5.2 | 19 ± 2.1 |
| PTH 2 ml                     | 210 ± 6   | 24.1 ± 2.6          | 1.51 ± .04 | 113 ± 4.4                 | 46 ± 2.9 | 34 ± 3.5 | 18 ± 1.3 |
| Homogenate at 23–30°, 42 hr† |           |                     |            |                           |          |          |          |
| Bonelli and Sala             |           |                     |            |                           |          |          |          |
| Control                      | 215 ± 10‡ | 5.1 ± 1.7           | 1.64 ± .10 | 18 ± 4.2                  |          | 18 ± 4.2 |          |
| PTH 1 ml                     | 200 ± 6   | 9.6 ± 1.5           | 1.52 ± .05 | 19 ± 2.2                  |          | 13 ± 3.4 |          |
| Kochakian and Reed           |           |                     |            |                           |          |          |          |
| Control                      | 209 ± 6   | 7.7 ± 1.7           | 1.52 ± .07 | 89 ± 3.9                  | 36 ± 2.1 | 29 ± 3.1 | 25 ± 1.7 |
| PTH 2 ml                     | 210 ± 6   | 24.1 ± 2.6          | 1.51 ± .04 | 75 ± 4.0                  | 34 ± 5.7 | 27 ± 4.9 | 22 ± 2.4 |

\* Autopsy 6.5 hr after inj. of parathyroid extract.

† Temperature and duration of autolysis.

‡ Stand. error of the mean,  $\sqrt{\frac{\sum d^2}{n(n-1)}}$ .

\* Publication No. 28, from the Oklahoma Medical Research Institute.

† Part of this investigation was aided by a grant

from the American Cancer Society.

‡ The parathyroid extract was provided by Eli Lilly and Co.