

available for investigational purposes.[†]

The results of this study, an earlier one, and unpublished data, suggest strongly that it is therapeutically feasible to raise the plasma protein values under the conditions outlined. The real elevation of the plasma albumin fraction and the total quantity of plasma albumin, due to an increase of the blood volume, appear significant. It is suggested by these data and similar observations on other patients being prepared for operation by feeding of a diet

[†] Provided by Armour and Company, Chicago.

rich in protein and carbohydrate and low in fat, that the intolerance to surgical trauma exhibited by such chronically wasted patients, is related to just such a contracted blood volume.

Conclusion. Feeding powdered bovine plasma protein to a patient made hypoproteinemic by a Meleney ulcer, was associated with an elevation of the plasma protein value. A large measure of this increase occurred in the albumin fraction. A daily intake of more than 500 g of protein was well tolerated by an ill patient.

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Effect of a Chromatin Derivative on the Healing of Skin Wounds.

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Experiments which are to be reported elsewhere showed that chromatin given intravenously produced a marked stimulation in the rate of mitosis in the regenerating liver of the rat.¹ We wish here to describe preliminary results obtained when a fraction derived from chromatin was applied to skin wounds.

Rat liver was perfused with 0.9% of NaCl and blended into 4 volumes of 0.9% of NaCl for 2 minutes in a Waring blender. Three-tenths molar NaOH was added during the blending in amounts sufficient to bring the pH to 7.4. The suspension was treated by the method of Claude and Potter² in order to isolate the filamentous substance they have called chromatin. The chromatin was lyophilized and extracted with boiling 50% acetone-50% alcohol. The fat-free chromatin was then extracted with 0.9% of NaCl. The

extract was then precipitated with acetone in which it was kept overnight. The clear supernatant solution had a bright yellow color. After decanting most of the acetone, the white precipitate was transferred aseptically to sterile test tubes in which it was lyophilized and sealed *in vacuo*. The 2.4 mg of dried material in each tube was taken up in 2 ml of saline for use on the rat wounds.

The wounds were made as described in the preceding paper. Controls were treated exactly as were the test animals, except that sterile saline was applied instead of the chromatin extract. With a hypodermic syringe, 2 or 3 drops were placed on the wound so that they formed one large drop filling the skin defect.[†] With a brush, a solution of methacrylate polymer in toluene was then applied over the drop and the adjacent normal skin. In the first 2 series of animals some of the wounds became infected. It was discovered that in these cases there had been leakage of the saline or test solution onto the normal skin so that the wound was not actually completely

* The work described in this paper was done under a contract recommended by the Committee on Medical Research between the Office of Scientific Research and Development and the University of California.

¹ Marshak, A., and Walker, A. C., unpublished.

² Claude, A., and Potter, J. S., *J. Exp. Med.*, 1943, **77**, 345.

[†] When the chromatin derivative was applied to wounds in which there was bleeding, the bleeding was stopped almost instantaneously.

sealed, although it seemed to be so on casual inspection. This difficulty was eliminated by painting the skin around the wound with the methacrylate polymer before the saline or test solution was applied. Following this procedure none of the wounds became infected. There was no evidence of irritation or inflammation following the application of the chromatin fraction.

For the first two series the following results were observed on the fifth postoperative day:

Granulation Tissue on the Fifth Post-Operative Day.		
	Control	Experimental
Granulation Absent	12	3*
Present	3 { $< \frac{1}{10}$ } { wound area }	13 { $\frac{1}{4}$ to $\frac{2}{3}$ } { wound area }
*Wounds badly infected.		

Of 15 controls only 3 showed any granulation on the fifth day. It appeared as a band 1 mm or less in width, about the periphery of the wound. On the other hand, only 3 of the 16 chromatin-treated wounds failed to show granulation tissue covering at least $\frac{1}{4}$ of the wound, and these 3 were badly infected.

In the third series in which none of the wounds became infected, no granulation appeared in the 4 control wounds until the fifth day. Of the 5 experimental animals, small islands of granulation appeared in 2 of them as early as the second day, in another on the third day, and by the fourth day all had granulation tissue over $\frac{1}{4}$ to $\frac{2}{3}$ of the wound

area. The granulation tissue in the experimental animals was bright red as compared with the pale red or pink of the controls and in microscopic section was clearly more vascular. Although the granulation tissue appears earlier in the treated wounds, the amount of such tissue formed is no greater than in the controls. After epithelialization was complete, the elevation of the treated wounds above the surrounding skin was no greater than in the controls.

Circumstances made it impossible to continue these experiments. However, the results obtained seem sufficiently promising to warrant further study.

We are indebted to Dr. E. L. Howes for testing the effect of whole chromatin on rabbit wounds and tissue cultures. In a personal communication he has reported that following intravenous administration of the chromatin, there was an optimum rate of healing of wounds of the rabbit's ear. This substance also increased the rate of growth of fibroblasts in tissue culture.

Summary. Following the application of a chromatin fraction to skin wounds of the rat, granulation tissue appeared one to three days earlier than in the controls. On the fifth postoperative day, several times as much of the surface of treated wounds was covered with granulation tissue as in the controls. There was no evidence of inflammation or irritation, and an excess of granulation tissue was not found.

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Healing of Experimental Skin Wounds.

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In the methods for the study of wound healing which have previously been described,

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there are inadequacies and sources of error which limit the usefulness of the procedures used. Carrel^{1,2} used the rate of closure of square skin defects as an index of healing,

¹ Carrel, A., *J. Exp. Med.*, 1913, **17**, 14.

² Carrel, A., and Hartman, A., *J. Exp. Med.*, 1916, **24**, 429.