TABLE II. Comparative Activity of Cathepsin III toward L-leucinamide and L-leucylpeptides.

Substrate	Preparation A			B			C		
	Enzyme conc.	$C_1 \times 10^4$	$C_0 \times 10^4$	Enzyme conc.	$C_1 \times 10^4$	$C_0 \times 10^4$	Enzyme conc.	$C_1 \times 10^4$	$C_0 \times 10^4$
L-leucinamide	.861	20	1.4	.202	76	7.3	.552	76	5.8
L-leucylglycine	.805	6.4	.60	.205	18	1.9	.565	18	1.8
L-leucyl-β-alanine	.852	3.9	.43	.205	13	1.4	.562	13	1.4
L-leucyl-γ-amino-butyric acid	.861	6.4	.63	.200	21	2.2	.567	18	1.8

Enzyme conc. = mg protein nitrogen/ml of test solution.
C_1 = First order specific reaction rate constant/enzyme conc.
C_0 = Zero " "

tide bond of the free carboxyl group of leucyl peptides. 2. The fact that L-leucinamide is hydrolyzed faster than are the peptides of leucine can possibly be explained by the hypothesis that the large groups attached to the peptide nitrogen of the substrates interfere with the formation of an enzyme-substrate complex. 3. The hydrolysis of the substrates by enzyme preparation A closely approximated zero order kinetics. The failure to observe a regular kinetic order for the reactions catalyzed by preparations B and C may be the result of destruction of the enzyme in the more highly purified preparations.

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Reversal of Cortisone Inhibition of Wound Healing by Tissue Culture Media.* (21194)

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The inhibition of normal wound healing by cortisone was demonstrated by Howes(1) and Spain(2). As early as 1911, Carrel demonstrated growth promoting and sustaining factors in embryo extracts as applied to tissue cultures of mammalian cells(3). The purpose of this paper is to present data on the influence of media containing Carrel's growth factors on cortisone inhibition of wound healing.

Methods. Under light ether anesthesia wounds 3 cm in length were incised through the skin to the fascia of the back of the neck of young adult male and female rats of the Sprague-Dawley strain. In one group single wounds were made. In a second group 2 parallel incisions 2 cm apart were made. The wounds were studied under 3 different conditions, as follows: 1) Wounds which were

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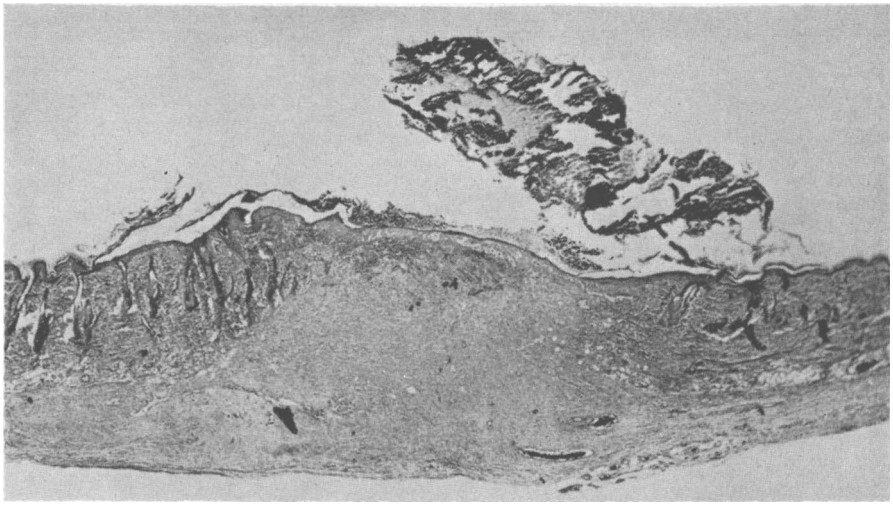

FIG. 1. Normal wound 8 days, 13½ ×, Mallory stain.

painted 10 times or injected twice daily with Hank's balanced salt solution were considered "normal wounds"; 2) Wounds produced on rats treated daily with a single subcutaneous injection of 10 mg of cortone acetate (Merck & Co.) were considered as "cortisone inhibited wounds"; 3) Wounds which were painted 10 times daily or injected twice daily with tissue culture media while the animals received the same dose of cortone acetate as depicted in 2) were considered as wounds showing reversal of cortisone inhibition of wound healing. Some of the wounds of groups 2) and 3) were inflicted in the same animal. The tissue culture media consisted of either defatted embryo extract prepared as a 50% solution in Hank's balanced salt solution or beef amniotic fluid obtained from a freshly slaughtered cow which was 2 months pregnant. The experiments extended over 6 to 14 days.

Results. Fig. 1 depicts a normally healing wound at 8 days. It shows normal granulation tissue formation permeated by moderate numbers of chronic inflammatory cells. The surface of the wound is covered by a scab of fibrin, dead leucocytes and red cells. All of the 14 normal wounds were similar in appearance.

Fig. 2 depicts a cortisone inhibited wound at 8 days. It shows virtually no granulation tissue or chronic inflammation. The scab appears the same as in Fig. 1. There were 35 cortisone inhibited wounds; all had the same appearance.

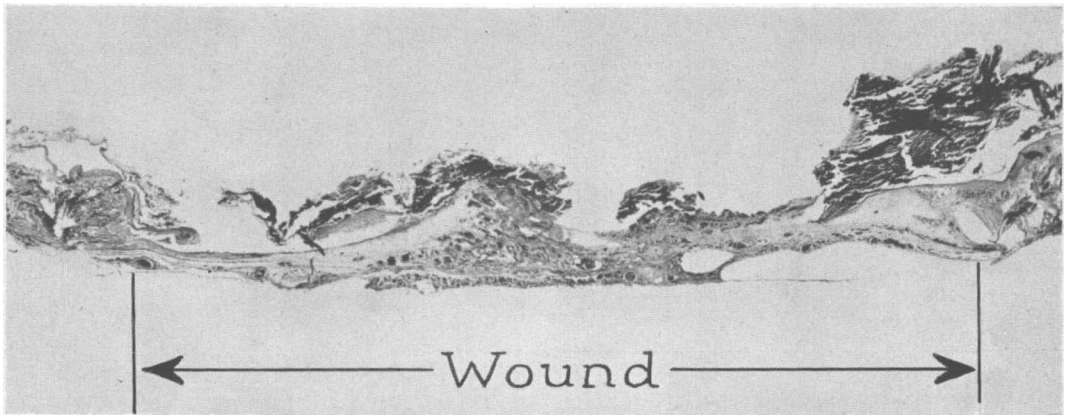

FIG. 2. Cortisone inhibited wound, 8 days, 13½ ×, Mallory stain.

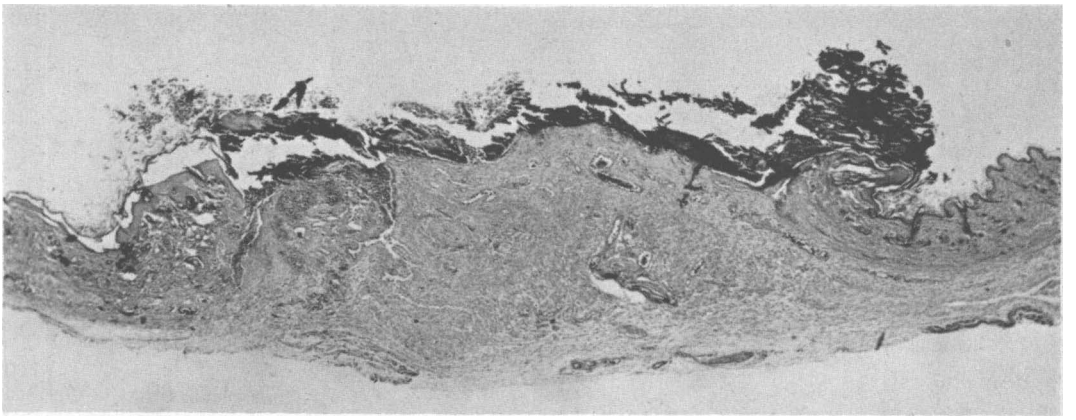

FIG. 3. Reversal of cortisone inhibition, 8 days, 13½ ×, Mallory stain.

Fig. 3 depicts the reversal of cortisone inhibition of wound healing by tissue culture media at 8 days. It shows abundant granulation tissue with a more pronounced deposition of collagen than present in the normal wound. The 32 treated wounds were of similar appearance.

Fig. 4 depicts a gross photograph illustrating systemic cortisone inhibition of wound healing on the left and reversal of cortisone inhibition on the right. Both wounds were 14 days old.

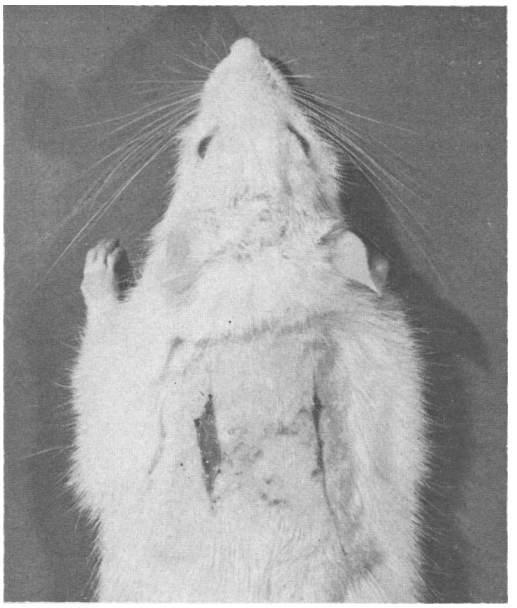

FIG. 4. Systemic cortisone inhibition—left. Reversal of cortisone inhibition—right. Both wounds 14 days old.

Three wounds show some escape from cortisone inhibition of wound healing. This is manifested by a moderate amount of granulation tissue and some chronic inflammation. These wounds are not comparable in their degree of healing to the normal nor the wounds treated with tissue culture media. Three wounds treated with tissue culture media fail to show complete reversal of cortisone inhibition of wound healing. These wounds resembled the wounds showing some escape from cortisone inhibition. The results are summarized in Table I.

The granulation tissues of these 3 types of wounds contain the following substances as demonstrated by a positive histochemical reaction: neutral fats, fatty acids, tri-glycerides, phospholipids, phosphatides, tyrosine, alkaline phosphatase, iron(4), non-specific esterase (5), potassium, free aldehyde groups, glycogen, calcium(4), free carbonyl groups(6), and protein bound sulfhydryl groups(7). Mast cells were present in equal numbers in all sections. These tests were less positive in the cortisone inhibited wounds because these wounds contain little or no granulation tissue.

Discussion. Other workers have indicated that the active growth promoting principle of embryo extract may be a pentosenucleoprotein (8-11). It is conceivable that the active component in the crude extracts used in these experiments is of a similar nature. If so these experiments lend some support to Carrel's hypothesis that chronic inflammatory cells liberate growth promoting substances which

TABLE I. Results of Wounds in Groups 1, 2 and 3.

Normal	14
Cortisone inhibited	35
Reversal of cortisone inhibition	32
Failure of complete cortisone inhibition	3
Failure of complete reversal of cortisone inhibition	3
Total	87

induce wound healing(12). Cortisone would then appear to function as an inhibitor of wound healing by virtue of its ability to modify inflammation(1,2).

Summary. 1. Local applications of tissue culture extracts will completely reverse cortisone inhibition of wound healing. 2. The granulation tissues of these wounds are histochemically similar.

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Adrenocortical Function in Hypo- and Hyperthyroidism.* (21195)

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Adrenal cortical hypertrophy following thyroid administration has been demonstrated many times since the work of Hoskins on the guinea pig(1). Its dependence on the presence of the pituitary was shown by Smith, Greenwood and Foster(2) in the course of metabolic studies with small doses of thyroxine in rats. Conversely, the cortical atrophy following thyroidectomy has frequently been noted (*e.g.* Evans, Simpson, and Pencharz (3)). The morphological picture of cortical atrophy resulting from thyroidectomy, and hypertrophy from treatment with thyroid hormone in the rat, has recently been illustrated by histological and cytochemical methods by Deane and Greep(4) and by Feldman(5). The question of the functional response of the adrenal cortex in hypothyroidism has been raised by Hill *et al.*(6) on the basis of impaired eosinopenic response to the ACTH test (Thorn *et al.*(7)) in certain hypothyroid patients and its improvement following thyroid hormone therapy in some of these patients. The ACTH test was adapted to use in experimental animals (dogs and rats) by Recant *et al.*(8). It offered a promising approach to the study of adrenocortical function in thyroid deficient and hyperthyroid rats. The work of Gabrilove and Soffer(9) on the ascorbic acid depletion response of propylthiouracil treated rats suggested another approach.

The plan followed in the present work was to study: A) the eosinopenic response to various stimuli such as ACTH or epinephrine, and B) the ascorbic acid depletion response to ACTH, in hypothyroid rats before and after thyroxine therapy, as compared with normal rats, and with normals rendered hyperthyroid by thyroxine administration.

Procedure. Male rats of the Long-Evans strain were surgically thyroidectomized at 21 days of age. After a prolonged interval, those which had shown a marked retardation in growth, and various external signs of thyroid deficiency (such as thinning of hair and accumulation of subcutaneous fat) were further screened for completeness of thyroidectomy

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