on the Friend ascites tumor cells are considered to substantiate these *in vitro* findings of Friend *et al.* The analysis of hemoglobin synthesis in pure tumor cell culture should provide the evidence and such studies are in progress in our laboratory.

Summary. Significant heme synthesis was observed in 2 Friend ascites tumor cell lines, when radioiron incorporation was measured and compared to Ehrlich ascites tumor cells and SN-36 ascites lymphoma cells.

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Effect of Lysosomal Labilizers and Pro-Inflammatory Substances on Connective Tissue Repair as Measured by Tensile Strength (32733)

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Many types of substances have been shown to influence wound healing. These include anti-inflammatory compounds, histamine depletors, nutritional additives, thyroxin, DL-penicillamine, zinc sulfate, deuterium oxide, cartilage powder, etc. (1–11). Few of these substances accelerate wound healing; most inhibit. Prudden *et al.* (12,13) have shown that the local application of bovine cartilage powder moderately increases wound tensile strength (WTS) on days 7 and 11 after wounding. This effect appears to be relatively specific, as other substances which promote growth of granulation tissue (e.g., talcum powder) do not accelerate healing and carrageenin actually reduces WTS (14). The increased strength of cartilage-treated wounds appears to be effected by some mechanism other than the laying down of increased numbers of collagen fibers (15) or by the sulfhydryl content of the cartilage (16).

The wound healing process is conveniently divided into (a) substrate phase (1–5 days); (b) collagen phase (5–15 days) and (c) maturation of scar phase (15 days) (17). The substrate phase is characterized by an acute inflammatory process whereas the collagen phase is associated with an increased tensile strength. The increased WTS is dependent upon both the amount and quality of collagen laid down (18, 19).

Glucocorticoids have been reported to inhibit both the inflammatory process and wound healing (20). Since a lysosomal mechanism may be involved in both of these processes, it was of interest to evaluate the influence on wound healing of substances which affect biological membranes. It was also of interest to determine if substances which augment the inflammatory reaction would likewise accelerate wound healing.

Materials and Methods. Male Wistar rats weighing 170 ± 10 gm were used in these studies. On day 1, the rats were anesthetized

with ether, the skin of the dorsolumbar region was shaved with electrical clippers and a wound approximately 1.5 inches in length was incised. The test materials were either administered systemically (daily) from the day of wounding or applied locally on the incised wound on day 1. The wounds were closed with 3 Michel clips. Eight rats from each group were killed and the skin WTS was measured on days 3, 9, and 15. The WTS was measured *in situ* according to the method previously described (20). All the materials tested, their respective doses and mode of administration are included in the tables.

Results. Locally applied papain, vitamin A acetate, digitonin, cartilage powder, hedera and aescin significantly increased WTS over that of controls at 3 days after wounding (Table I). The effects of these compounds were less evident or absent on days 9 and 15 after wounding. On day 9, digitonin enhanced WTS at 1, 4, 8 mg/rat; higher doses were less effective and significant inhibition of healing was observed on day 15 (Table I). All the saponins tested (Table II) significantly increased WTS at 3 days. Systemically administered estrogens such as ethynyl-

TABLE I. The Effect of Locally Applied Papain, Vitamin A Acetate, and Digitonin on Rat Skin Wounds.

	Dose (mg/rat)	Wound tensile strength (% change from controls) 3 Days	9 Days	15 Days
1. Papain	5	+28	+13	+13[a]
2.	25	+40[a]	+11	+12[a]
3.	50	+58[a]	+12	+17[a]
4.	100	+52[a]	+10	− 3
5.	200	+52[a]	− 4	− 7
6. Vitamin A	5	+42[a]	+11	+ 3
7.	25	+56[a]	+14	+22[a]
8.	50	+43[a]	+22[a]	+ 8
9.	100	+56[a]	+ 2	+23[a]
10.	200	+38[a]	0	+16[a]
11. Digitonin	1	+56[a]	+14[a]	+17[a]
12.	4	+102[a]	+22[a]	+14[a]
13.	8	+118[a]	+16[a]	+ 2
14	16	+148[a]	+11	−22[a]
15.	32	+200[a]	—	−17[a]

[a] $p \leq .05$.

TABLE II. Effect of Some Saponins on Rat Skin Wounds.

	Dose (mg/rat)	Wound tensile strength (% change from controls) 3 Days
1. Cartilage powder[b]	5	+16
2.	50	+33[a]
3. Aescin[c]	5	+133[a]
4.	50	+136[a]
5. Acidified aescin	50	+215[a]
6. Saponin[d]	5	+153[a]
7.	50	+135[a]

[a] $p \leq .05$.

[b] Obtained from Nutritional Biochemicals Company, Cleveland, Ohio.

[c] A saponin from horse chestnut *Aesculus hippocastanum.*

[d] A saponin, probably derived from a variety *Hedera helix* L. Obtained from Dr. Madaus and Co., West Germany.

estradiol-3-cyclopentyl enol ether (EECPE), estradiol cyclopentyl propionate (ECP), Premarin,[1] and thyroactive compounds, 3,3'5-triiodo-*l*-thyronine, 3,3'5-triiodothyropropionic acid, *l*-thyroxin and 3,3'5-triiodothyroacetic acid reduced WTS on days 3 and/or 9 (Table III). The noncalorigenic thyroxin analogues were without effect at the dose tested (which were equivalent to or higher than those of the thyroactive substances). Locally applied carrageenin, diethylstilbestrol, and estradiol-17β inhibited WTS whereas growth hormone and deoxycorticosterone acetate (DCA) did not influence WTS at the dose level employed (Table IV).

Discussion. Wound healing appeared to be accelerated by several types of substances previously reported to labilize membrane systems (21–25). Thus local application of digitonin, vitamin A acetate, papain, hedera, aescin and other saponins to the freshly incised wound resulted in an increase in WTS as measured on day 3 after wounding. (This effect was less pronounced 9 and 15 days later.) Previous work showed that substances such as glucocorticoids which stabilize lyso-

[1] Premarin®—conjugated equine estrogens.

TABLE III. Effect of Some Estrogens and Thyroxin Analogues on Rat Skin Wounds.

Compounds	Dose (μg per 0.2 ml per rat)	Route of adm.	Wound tensile strength (% change from controls) 3 Days	9 Days
1. Premarin	10	po[c]		— 9
2.	100	po[c]		—19[a]
3. Ethynylestradiol-3-cyclopentyl enol ether (EECPE)	1	po[c]		—13
4.	10	po[c]		—21[a]
5. Estradiol cyclopentyl propionate (ECP)	1	sc[c]		— 3
6.	5	sc[c]		—19[a]
7.	50	sc[c]		—28[a]
8. *l*-Thyroxine	40	sc[b]	0	—19[a]
9. 3,3'5-Triiodo-*l*-thyronine	8	sc[b]	—19[a]	—20[a]
10. 3,3'5-Triiodothyroacetic acid	200	sc[b]	— 4	—19[a]
11. 3,3'5-Triiodothyropropionic acid	300	sc[b]	—22[a]	—10[a]
12. 3,3'5'-Triiodo-*dl*-thyronine	450	sc[b]	— 2	— 4
13. 3,3'5'-Triiodothyropropionic acid	500	sc[b]	— 4	— 2

[a] $p \leq .05$.

[b] Doses for thyroxine and its analogues are approximately equivalent on a calorigenic basis.

[c] The EECPE and ECP were dissolved in sesame oil and administered to ovariectomized animals 1×/week × 3 starting 1 week prior to wounding whereas Premarin, which is water soluble, was administered daily.

TABLE IV. Effect of Various Substances on Rat Skin Wounds.

	Dose (mg/rat)	Route of adm.	Wound tensile strength (% change from controls) 7 Days	9 Days
1. Carrageenin	10	Local		—24[a]
2. Ascorbic acid	100	Local	— 8	
3. DCA	50	Local	—14	
4. Chlorpromazine	25	Local		—27[a]
5. Diethylstilbestrol	10	Local	—20[a]	
6. Estradiol-17β	5	Local	—28[a]	
7. Beef growth hormone (NIH-GH-B7)	2.5	sc		+ 9

[a] $p \leq .05$.

somal membranes inhibit wound healing (1). On the other hand, high doses of digitonin were found to depress rather than to stimulate wound healing. Locally applied estradiol-17β, which reportedly is a disruptor of lysosomal membranes *in vitro* (21) retarded wound healing in the present study. Thus it would be an oversimplification to postulate that wound healing might be controlled through chemical regulation of lysosomal particles. Estrogens are known to stimulate the activity of degradative enzymes (26) such as acid phosphatase (27) and to enhance collagen synthesis (28). However, it may be argued that estrogens also stimulate the pituitary–adrenal axis (29), increase phagocytosis (30) and inhibit growth of fibroblasts (31). Each of these latter functions would theoretically oppose the wound healing process as currently understood.

Similarly, it is impossible to generalize that pro-inflammatory agents are wound healing accelerants. Present work confirms the previous report (14) that granuloma-producing agents such as carrageenin inhibit rather than accelerate wound healing. Also systemically administered *l*-thyroxin and other calorigenically active thyroxin analogues depressed rather than augmented WTS. Yet thyroactive agents are known to enhance the acute inflammatory response (32) perhaps by favoring histamine synthesis (33) and edema formation (32). The picture is confusing because thyroactive agents may also trigger the pituitary–adrenal axis (34), may enhance phagocytosis (30) and suppress the secretion of TSH, a known inhibitor of wound healing (7). Noncalorigenic thyroxin analogues were inactive and thus did not provide a possible clue as to mechanism. Growth hormone, a known stimulator of fibroblastic proliferation and DCA, which according to Rindani (35), is a pro-inflammatory agent, likewise failed to stimulate wound healing in the present study. Cartilage powder, which we confirmed to promote wound healing, is not an inflammatory substance. Thus, although there is present an obvious inflammatory reaction during the substrate phase of wound healing (17) and demonstrably enhanced activity of lysosomal enzymes at the edges of a fresh wound (36), it would be contrary to the data in hand to postulate that successful repair of connective tissue is dependent upon either a full blown inflammatory process or upon the release of lysosomal enzymes per se. Indeed, the evidence suggests that the processes of inflammation and healing may be separable: that agents may be found which favor repair under conditions of minimal inflammation.

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