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CHLOROPHYLL*

AN EXPERIMENTAL STUDY OF ITS WATER SOLUBLE DERIVATIVES IN WOUND HEALING

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THE problem of wound healing becomes of paramount importance in time of war. In the intervals between wars somewhat desultory and fragmentary investigations continue to be carried on in this general field, but the bulk of research is directed at other more spectacular issues of current interest. The prosaic, commonplace problem of the repair of wounds, like the proverbial poor relative, is always with us, and is dismissed as one of little interest or likelihood of acclaim. In the past two or three years, however, medical literature has devoted a very considerable percentage of its space to studies relating to the treatment of burns and wounds. It is obviously impossible for any one individual to familiarize himself with more than a small fraction of this literature, much less carry out in his own practice the thousand and one recommendations for the treatment of such injuries.

For that reason, it is well to adopt a conservative and frankly skeptical point of view regarding the relatively extravagant claims of each enthusiastic contributor to the rapidly growing chemotherapeutic armamentarium available for the treatment of burns and other traumatic injuries. Such publications as the recent National Research Council's Military Surgical Manual No. 5[20] reflect this sane approach. Likewise, the contributions of Bowers,[6] Whipple,[28] Waugh,[27] Harkins[16] and Brush and Lam[7] to this field present a most encouraging and hopeful attitude. They all stress the basic physiological principles involved in the healing process which have been all too frequently disregarded until comparatively recently, although Carrel[9] and his associates over twenty-five years ago recognized their importance and evolved certain fundamental laws in respect to wound healing.

These earlier investigators developed a mathematical formula of geometric progression in respect to the estimated time required for repair of any wound, based on its surface area. They recognized that larger wounds tended to heal more rapidly than small ones; that the repair phenomenon was influenced by age, tissues from older individuals requiring longer to heal than those of children. They noted the so-called "lag" or latent period between the time the injury occurred and the beginning repair phenomenon, which is still credited to the biophysical changes associated with the inflammatory exudative phase of the process. And it was Carrel who evolved the "trephone" theory of enzymatic growth stimulating factors being produced through tissue destruction or inflammatory cell metabolism. "Laudable pus" was explained on this same basis, as a necessary irritant to induce cell proliferation, for in its absence, repair was retarded or ceased altogether.

Menkin's[19] now classical studies on inflammation have gone a long way to explain these various phenomena by sound physicochemical theory; stressing the importance of the hydrogen ion concentration of the tissues, the rôle of fibrinolysin, the place of the euglobulin fraction of the exudate (necrosin) which appears to be responsible for the subsequent course of events following the initial injury, and the

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value of the pseudoglobulin chemotactic fraction (leukotaxin). Tissue culture studies have emphasized the importance of these metabolic or breakdown enzymic products of tissue injury as evidenced by the routine use of such tissue extracts as splenic extract, embryonic tissue juice, "leucocytic cream" and the like as an integral part of the usual media employed.

Currently, a good deal of interest has been shown in a product devised by Sperti[25] and his associates for the treatment of minor burns and injuries, which they have termed "Bio-dyne" ointment. Its use is predicated on the same fundamental theory that cells liberate a growth stimulating factor when injured or destroyed. These workers have employed yeast cells injured or destroyed by ultraviolet irradiation as the source of their growth stimulating factor or "hormone." Cook and Fardon[11] and Nutini[21] from the same laboratories review the concept of wound hormones from the time of Virchow's "formative stimulus" in 1858, and stress the evidence which they have obtained experimentally of the production by injured cells of substances which "promote the proliferation and metabolism of living cells."

In our own studies we have noted the marked "boosting" effect of leucocytic cream extract on cell growth in tissue cultures.[24] We believe the evidence is convincing that some substance or substances derived from injured or dead tissue or inflammatory cells are the chief factors responsible for initiating the reparative phase of any local destructive process regardless of the etiologic agent producing the immediate injury. It appears equally apparent that the so-called lag period before repair ordinarily begins is dependent upon the catabolic cell changes resulting from the injury. Thus, the entire exudative and reparative phases of tissue injury might be thought of in terms of a series of complicated chemical reactions which undoubtedly in due course of time can be reduced to mathematical equations. One gains the impression that the reparative phase of wound healing is dependent upon the accumulation of an adequate amount of the growth stimulating factors to neutralize and hold in check the catabolic phenomena. Whether this mechanism is primarily dependent upon the liberation of these stimulating factors quantitatively in respect to the number of cells injured or destroyed, or whether these products are actually enzymes or "hormones" capable of propagation interstitially in the tissue fluids is uncertain and perhaps not strictly pertinent to the problem at this time.

In any event, therapeutic efforts to reduce this lag period either by diminishing the catabolic phase or by stimulating the anabolic, proliferative processes are the objective desideratum of all investigators. It is apparent that gross bacterial infection delays healing, so that one aim of any treatment is to produce bacteriostasis. At the same time it is not at all certain that complete bacterial sterilization is either necessary or even advisable, for it is quite possible that minimal saprophytic surface contamination may augment the reparative proliferation by supplementing the necessary stimulating factor with the breakdown of the bacteria themselves. It is equally obvious that a poor blood supply as occurs in chronic indolent ulcers, especially those associated with *x*-ray burns or on a varicose vein basis is another important factor. In these latter conditions, a slowly developing fibrosis with resultant hyalinization of the collagen plays an important part in the retardation of the repair process through further cutting down the blood supply.

With these theoretical considerations in mind, we have joined the vast group of investigators in this field in an attempt to add our contribution toward the solution of the problem. In view of the considerable discussion which has been raised in the past few years regarding the possible place of chlorophyll in our therapeutic armamentarium, we have undertaken a study on wound healing using water soluble chlorophyll preparations made up in various ways

(as solutions, jellies, and ointments) in the treatment of experimentally induced burns and wounds in rats, guinea pigs, rabbits and dogs, as well as in a limited number of clinical cases.

In order properly to evaluate the value of chlorophyll* therapeutically, a considerable number of other agents were studied in identical manner, using standard surgically induced wounds or dry heat burns. The agents† employed were the following:

1. Chlorophyll—0.2 per cent alkaline saline solution (pH 7.3–7.8)
2. Chlorophyll—0.2 per cent acid buffered solution (pH 6.6–6.8)
3. Chlorophyll—lanoline base ointment—0.5, 1.0, 2.0 and 3.0 per cent
4. Chlorophyll—petrolatum-cholesterol base ointment—2.0 per cent
5. Chlorophyll—hydrophilic base jelly—1.0 per cent
6. "Bio-dyne" ointment
7. Vitamin B complex ointment—1 per cent
8. Vitamin C ointment—0.1 per cent
9. Vitamin D (cod-liver oil) ointment
10. Methionine ointment—0.05 per cent
11. Castilian malva used as 10 per cent infusion
12. Sulfanilamide (powdered)
13. Sulfathiazole (powdered)
14. Sulfathiazole ointment—5 per cent
15. Sulfadiazine spray—2 per cent
16. Scharlach R ointment
17. Tetrodine dusting powder—6 per cent iodine
18. Controls—untreated

* Whenever the term "chlorophyll" is used in these studies, the water-soluble derivatives are meant, the term "chlorophyll" being used solely for the sake of brevity.

† Acknowledgments: *Agents No. 1 to 5*—The experimental aqueous soluble chlorophyll products have been generously supplied us through the courtesy of the Rystan Company of New York, sole appointee of the Lakeland Foundation of Chicago, in accordance with the regulations of the Federal Food, Drug and Cosmetic Act relative to therapeutic products. *Agent No. 6*—The Bio-dyne ointment was kindly supplied us by the Sperti Laboratories of Cincinnati. *Agent No. 9*—The cod liver oil ointment (Gadamont—E. L. Patch Co.) was purchased in the open market. *Agent No. 10*—The methionine was supplied for experimental study by Merck & Company of Rahway, N.J., and the other Merck products used were made available through our own pharmacy. *Agent No. 11*—The Castilian Malva was supplied us by the Upjohn Company of Kalamazoo, Michigan. *Agent No. 17*—The Tetrodine dusting powder was supplied us by the Tyler Laboratories of Brooklyn, N.Y.

PROCEDURE

In the smaller animals—rats, guinea pigs and rabbits—under nembutal anesthesia, symmetrical, roughly circular, 1.0 cm. areas of skin were excised from the thorax, abdomen or back, after first shaving and painting the operative field with tetrodine. In the rats, guinea pigs and rabbits, a single pair of lesions was produced. As in Clark's recently reported studies,[10] the left side was regularly used as the control, the right side for the experimental treatment. In the dogs it was possible to make as many as four pairs of 2.5 cm. lesions. In these animals the anterior pair of lesions were used as controls, the left, right and posterior pairs as the experimental areas. The procedure varied somewhat in this group inasmuch as it has been impossible to obtain dogs in the numbers desired. Thus as many as three test agents might be employed simultaneously on the same animal. This is open to the criticism that the amount of systemic reponse from absorption of the various agents is difficult to evaluate and there might therefore be a cumulative effect. However, it is believed that this criticism can be satisfactorily answered, as in no instance was such multiple testing employed without also testing a control animal in which only a single agent was used. The entire method is relatively crude but follows the pattern employed by other investigators in this field such as Whipple,[28] Thompson[26] and Ravdin, Harkins,[16] Brush and Lam,[7] Clark,[10] Boehringer,[5] Buergi[8] and Gruskin.[14] It is dependent upon observing the difference in the rate of healing of standard experimentally induced wounds in a sufficient number of cases to render the results statistically significant.

In the larger animals—dogs—a technic was evolved whereby wounds identical in size could be produced by mounting a Bard Parker knife blade in an adjustable arm attached to a spindle. The spindle has three pinpoints in its foot which hold the instrument in position on the skin surface

with but slight pressure. By revolving the arm, a perfect circle can be cut through the skin and subcutaneous tissues down to the fascial layers. This disc of skin can then be readily excised sterilely leaving a clean-based circular defect of whatever diameter is desired—in these particular animals, 2.5 cm. A cork or aluminum ring somewhat larger than the lesion is then cemented to the skin with celloidin, lucite or Johnson & Johnson's liquid adhesive. This enables us to treat the lesions individually with no danger of the test agent contaminating the control area. The test agent is introduced generously on a small piece of double-thickness coarse meshed surgical gauze cut to fit just inside the protecting ring. The intervening areas are loosely packed with crumpled gauze and a binder made of light weight duck applied over the whole trunk. This is held in place by cutting two holes for the forelegs, overlapping the two ends of the binder and sewing them snugly over the back of the animal with interrupted stitches. To protect the field further from harm, a wide collar (6 to 8 inch radius) made of two layers of corrugated cardboard with the corrugations at right angles to each other to prevent its buckling is placed around the dog's neck, and each dog put in an individual cage. Even with these precautions, an occasional dog may get his dressing off, apparently by persistent rubbing against the cage, but in general, the method seems the most satisfactory we have been able to discover.

By this technic, it is comparatively easy to redress the wounds as desired, usually every second or third day. At each examination the extent of healing is recorded by measuring the remaining unepithelialized area, either by using Brush and Lam's technic of actually tracing the edges of the wound on cellophane or by the use of calipers in three transverse directions. It is not believed that planigraphy is necessary, as our chief concern is in respect to the comparative time required to secure complete healing in each case.

This method has the advantage, as Clark has emphasized, of permitting regulated infection to be introduced into the experimental field without much likelihood of contamination of the control area, and thereby making it possible to test the bactericidal or bacteriostatic effect of various agents *in vivo* in a somewhat crude, but at least, comparable manner. The second group of experiments reported here deals with this phase of the problem.

Finally, a similar set of experiments was carried out in which standardized wounds, produced by dry heat, were studied in respect to their healing characteristics. For this purpose, steel cylinders about 2.5 cm. in length and of the diameters desired—2.5 and 5.0 cm., respectively—were hollowed out at one end to permit introducing a flanged tube. This tube serves the double purpose of acting as a handle, and of permitting the introduction of a thermometer into the hollow cylinder. The hollow cavity is filled with mercury to prevent any insulation in respect to the thermometer. The apparatus is then heated to the desired temperature and applied to the skin surface without other pressure than its own, known weight for varying lengths of time, depending on the degree of burn desired. From long experience we have found that there is very little heat loss—roughly 5°C. for each fifteen seconds of application with the smaller, and about 2°C. loss with the larger cylinder over the same time period. For practical purposes, we have found 250°C. applied for thirty seconds gives a very satisfactory mild third degree burn in dogs. As in the case of the surgically produced wounds the lesions are uniform and completely comparable for the purposes of a study such as this. The skin sloughs off in four to five days, leaving a clean-based, circular, ulcerated surface. By protecting the burned areas in the same way as in the preceding surgically induced wounds they can be kept free of bacterial contamination or not, as desired, and topical application of any of the medicaments under study can be carried out satisfactorily.

The experiments included in this report fall into three main groups as already indicated: (1) The healing of experimentally produced clean surgical wounds; (2) the healing of experimentally infected surgical wounds, and (3) the healing of experimentally induced dry heat burns.

TABLE I
STATISTICAL SUMMARY OF EXPERIMENTAL MATERIAL

	No. of Animals	No. of Lesions	No. Control Lesions	No. Test Lesions
Group I				
Sterile Wounds				
Experiments 1–18 Rats	108	216	114	102
19–36 Guinea pigs	108	216	114	102
37–54 Rabbits	78	156	82	74
55–72 Dogs	76	608	164	444
Group II				
Infected Wounds				
Experiments 73–90 Guinea pigs	208	416	212	204
91–108 Dogs	36	254	84	170
Goup III				
Third Degree Burns				
Experiments 109–126 Dogs	48	384	108	276
Totals	662	2,250	878	1,372

In each of these three main groups there are eighteen separate experiments with each type of animal used, based on the seventeen preparations under study plus a supplementary control group. In the small animals a single test area has been used with a corresponding control area on the opposite side of the animal. In the dogs eight lesions have been produced, two of which have served as controls, and six as test areas. Table I shows in summary form the statistical data relating to the number of experiments, number of animals used, the number of control lesions and the number of experimental lesions tested.

The seventeen preparations tested can be reduced in actual number to eight, by combining the several chlorophyll, vitamin and sulfone compounds as group products. Justification for such simplification of the accumulated data is seen in the similarity of results within these related group preparations. In the simple, clean, wound healing experiments, identical studies were carried out on rats, guinea pigs, rabbits and dogs. In Group II, consisting of experimentally induced infected wounds only guinea pigs and dogs were used, and in the Group III burn experiment dogs were found to be much more satisfactory to work with than any of the smaller animals.

TABLE II
1 PER CENT CHLOROPHYLL IN HYDROPHILIC JELLY BASE
Time Required for Healing 1.0 Cm. Sterile, Surgically Produced Wounds, Expressed in Days

Identification		Control Area	Test Area	Summary
Rat	31	12	8	
	32	16	12	Accelerated....4—66.7%
	33	16	10	Unaffected.....2—33.3%
	34	14	14	Delayed.....0— 0.0
	35	10	8	
	36	14	14	
Guinea Pig	31	16	10	
	32	18	10	Accelerated....5—83.3%
	33	14	14	Unaffected.....1—16.7%
	34	14	8	Delayed.....0— 0.0
	35	12	10	
	36	14	10	
Rabbit	31	16	12	
	32	14	14	Accelerated....5—83.3%
	33	16	10	Unaffected.....1—16.7%
	34	10	8	Delayed.....0— 0.0
	35	16	12	
	36	12	8	
Total		254	192	Accelerated...14—77.7%
				Unaffected.....4—22.3%
Average		14.1	10.6	Delayed.....0— 0.0

EXPERIMENTAL RESULTS

The experimental results are probably best presented for consideration through the following series of tables in which the statistical data have been assembled for comparative study and analysis. Such additional comment or discussion as seems pertinent to an understanding of the figures is included. It does not seem necessary or even advisable to include the individual protocols of all the experiments as the useful information is adequately summarized in the combined tables. However, representative protocols of a couple of typical experiments are presented to illustrate the method whereby the data as a whole were obtained for statistical analysis. (Tables II and III.)

In the first group of experiments, which deals with the healing of clean, surgically produced wounds, the complete data will be found summarized in Table IV, as these relate to 1.0 cm. wounds in small animals, and to larger 2.5 cm. wounds in dogs, respectively.

Not only is the percentage of such accelerated healing notably better than with any of the other agents studied, but the average time interval required for complete healing decreased by 3.5 days (from 14.1 to 10.6 days) a figure just short of 25 per cent (24.9 per cent). In a considerable number of the animals the differential time interval was as much as six to eight days which is a very real difference. When the reading is only a matter of two days, (the routine time interval between redressing and examining the wounds), as Brush and Lam have emphasized, one is justified in querying any actual effect of the agent under study. But when the time required for healing is reduced by one-fourth in from two-thirds to three-quarters of a series of over four hundred lesions, it would seem to indicate that chlorophyll does cause some biologic response in respect to stimulating cell growth which can be put to a useful

TABLE III
1 PER CENT CHLOROPHYLL IN LANOLINE OINTMENT BASE
TIME REQUIRD FORE HEALING OF 2.5 CM. STERILE, SURGICALLY PRODUCED WOUNDS IN DOGS EXPRESSED IN DAYS

	Dog No.	Total No. Lesions	No. of Control Lesions	No. of Test Lesions	Time of Healing in Days: Control		Test Areas						Average: Control	Average: Test
Experiment No. 57 Test animals.	9	8	2	6	16	18	10	8	8	12	10	10	17	9.6
	10	8	2	6	14	12	12	12	14	14	12	14	13	13.0
	11	8	2	6	16	14	12	12	10	12	14	12	15	11.6
	12	8	2	6	12	12	12	14	12	12	20*	22*	12	15.3
	13	8	2	6	16	14	10	12	12	12	10	12	15	11.3
	14	8	2	6	14	14	8	12	10	12	14	12	14	11.3
Experiment No. 72 Control animals.	69	8	8	..	12	14	14	12	14	14	12	14	13.2	
	70	8	8	..	16	14	18	14	16	16	18	14	15.7	
	71	8	8	..	14	16	12	12	12	16	14	14	13.7	
	72	8	8	..	12	12	14	16	14	12	12	14	13.2	
Total	10	80	44	36	..	..	..	..	..	..	...	...	14.0	12.1

Summary: Healing accelerated—4 animals or 67.0%
" unaffected —1 animal or 16.5%
" delayed —1 animal or 16.5%

* Infected.

In summary, we note that 67 per cent of all the wounds treated by one or another preparation of chlorophyll healed more rapidly than their controls. This percentage varies from 55 per cent in the case of the acid buffered aqueous solution to 75 per cent with the 1 per cent hydrophylic base jelly as a vehicle. Referring to Table II we see that, using the hydrophilic jelly preparation, as high as 83.3 per cent of the wounds in guinea pigs and rabbits showed such acceleration in the healing process.

purpose in the many problems associated with wound healing.

This stands out particularly prominently if one summarizes the results obtained with various vitamin and sulfone compounds (Table v), as well as with the various miscellaneous agents tested by ourselves and by Brush and Lam in which it can be seen at a glance that little or no favorable effect upon the rate of healing itself actually bactericidal, but that it does exert a definite bacteriostatic effect *in vitro*, apparently through its oxidative action. Its mode of action *in vivo* is still obscure, but it is believed that interference with fibrinolysin production and its growth stimulating capacity are also of importance.

In the second group of experiments which deals with artificially infected surgically produced wounds, the data in summary

TABLE IV

HEALING OF STERILE, SURGICALLY INDUCED WOUNDS

	Rats					Guinea-Pigs					Rabbits					Dogs					Summary							
		Healing					Healing					Healing					Healing						Healing					
	No. Animals	Accelerated	Unaffected	Delayed		No. Animals	Accelerated	Unaffected	Delayed		No. Animals	Accelerated	Unaffected	Delayed		No. Animals	Accelerated	Unaffected	Delayed		Total Number of Animals	Number Accelerated	Accelerated Per Cent	Number Unaffected	Unaffected Per Cent	Number Delayed	Delayed Per Cent
Chlorophyll—alkaline solution	6	4	1	1*		6	3	2	1*		4	2	1	1		4	3	1	0		20	12	60	5	25	3	15
Chlorophyll—acid solution	6	4	2	0		6	4	1	1*		4	1	3	0		4	2	2	0		20	11	55	8	40	1	5
Chlorophyll—lanoline ointment	6	4	2	0		6	4	2	0		6	4	1	1*		6	4	1	1*		24	16	67	6	25	2	8
Chlorophyll—cholesterol ointment	6	5	1	0		6	4	2	0		6	4	2	0		6	5	1	0		24	18	75	6	25	0	0
Chlorophyll—hydrophilic jelly	6	4	2	0		6	5	1	0		6	5	1	0		6	4	2	0		24	18	75	6	25	0	0
Total chlorophyll	30	21	8	1*		30	20	8	2		26	16	8	2		26	18	7	1		112	75	67	31	27.5	6	5.5
Bio-dyne	6	2	3	1*		6	2	4	0		4	2	2	0		4	1	3	0		20	7	35	12	60	1	5
Vitamin B ointment	6	1	4	1		6	0	6	0		4	1	3	0		4	0	3	1*		20	2	10	16	80	2	10
Vitamin C ointment	6	2	3	1*		6	1	4	1		4	0	4	0		4	1	3	0		20	4	20	14	70	2	10
Vitamin D ointment	6	2	4	0		6	2	4	0		4	1	2	1*		4	0	3	1		20	5	25	13	65	2	10
Methionine ointment	6	3	2	1		6	2	4	0		4	2	2	0		4	1	3	0		20	8	40	11	55	1	5
Castilian malva infusion	6	1	4	1*		6	2	3	1*		4	1	3	0		4	2	2	0		20	6	30	12	60	2	10
Sulfanilamide powder	6	0	2	4		6	0	3	3		4	0	2	2		4	0	1	3		20	0	0	8	40	12	60
Sulfathiazole powder	6	0	3	3		6	0	2	4		4	0	3	1		4	0	2	2		20	0	0	10	50	10	50
Sulfathiazole ointment	6	0	2	4		6	1	3	2		4	0	1	3		4	0	2	2		20	1	5	8	40	11	55
Sulfadiazine spray	6	1	4	1		6	0	4	2*		4	1	1	2		4	0	1	3		20	2	10	10	50	8	40
Scharlach R ointment	6	2	3	1*		6	3	3	0		4	1	3	0		4	1	2	1		20	7	35	11	55	2	10
Tetrodine powder	6	1	3	2		6	0	4	2		4	0	2	2		4	0	3	1		20	1	5	12	60	7	35
Control	6	..	..	..		6	..	..	..		4	..	..	..		2	..	..	..		18						
Totals	108	..	..	..		108	..	..	..		78	..	..	..		76	..	..	..		370						

* Infection.

was obtained, and indeed, in a not insignificant percentage of the wounds there was an appreciable retardation noted in the rate of healing. As a matter of collateral interest, it should be mentioned that where actual delay in healing occurred in the animals treated with chlorophyll there was secondary infection present. That aspect of the problem is more clearly seen in the second group of experiments in which infection was introduced into the wounds. It has already been brought out in another paper of this series[24a] that chlorophyll is not of form are presented in Table VI. Here again, it is to be noted that almost the same relative acceleration in healing is noted in the chlorophyll group as compared with the other test agents as in the uninfected series of cases. However, in the group of miscellaneous agents, several of which are more or less antiseptic in their action, there is a definite shift in the picture toward more rapid healing with a corresponding drop in the delayed healing percentage figures. This is particularly striking in the case of "Tetrodine," a stable aqueous

preparation of iodine, as well as in the various sulfone compound treated lesions. Of the three sulfa drugs tested in this respect, sulfadiazine in a 2 per cent spray seemed the most effective in controlling infection, and thereby, indirectly hastening repair. Bio-dyne, here as in the clean wounds appeared to have an appreciable accelerating effect from the percentage standpoint, but actually this difference was hard to evaluate, because it was only by a matter of a couple of days in the majority of cases. Scharlach R ointment in our hands had little or no effect on the rate of growth. This independently confirmed the report by Brush and Lam. The same inconclusive evidence was obtained in respect to the use of methionine and the various vitamin ointments as well as Castilian malva infusion preparations so far as their effect upon wound healing is concerned. (Table VII.)

In the third group of experiments which were designed to show the healing effect of topical applications of various agents on standard dry heat burns of varying size and

TABLE V

RATE OF HEALING OF EXPERIMENTAL SURGICAL WOUNDS

Test Agent	Accelerated, Per Cent	Unaffected, Per Cent	Delayed, Per Cent
Chlorophyll preparations	67.0	27.5	5.5
Vitamin ointments	18.4	71.6	6.0
Sulfa compounds	3.8	45.0	51.2
*Miscellaneous	19.4	55.1	25.5

* See Table IV this article and Table II in Brush and Lam's (loc. cit.).

TABLE VI

HEALING OF SURGICALLY INDUCED WOUNDS, EXPERIMENTALLY INFECTED WITH 0.5 CC. OF A MIXTURE OF STAPHYLOCOCCUS AUREUS (STRAIN C-209) AND STREPTOCOCCUS HEMOLYTICUS (LANCEFIELD GROUP A STRAIN C-203) TWENTY-FOUR HOUR BROTH CULTURES

Test Agent	Guinea Pigs				Dogs					Summary					
		Healing					Healing				Healing				
	No. Animals	Accelerated	Unaffected	Delayed	No. Animals	No. Lesions Treated	Accelerated	Unaffected	Delayed	Total Lesions	Accelerated	Per Cent	Unaffected	Per Cent	Delayed	Per Cent
Chlorophyll—alkaline solution	12	8	2	2	2	4	3	1	0	16	11	69	3	19	2	12
Chlorophyll—acid solution	12	9	2	1	2	4	3	1	0	16	12	75	3	19	1	6
Chlorophyll—lanoline ointment	12	6	4	2	2	6	4	1	1	18	10	55	5	28	3	17
Chlorophyll—cholesterol ointment	12	5	6	1	2	6	4	2	0	18	9	50	8	44	1	6
Chlorophyll—hydrophilic jelly	12	7	4	1	2	6	5	1	0	18	12	67	5	26	1	7
Total chlorophyll	60	35	18	7	10	26	19	6	1	86	54	63	24	28	8	9
Bio-dyne ointment	12	4	6	2	2	12	5	6	1	24	9	38	12	50	3	12
Vitamin B ointment	12	2	10	0	2	12	3	8	1	24	5	21	16	67	3	12
Vitamin C ointment	12	1	10	1	2	12	2	9	1	24	3	13	19	79	2	8
Vitamin D ointment	12	3	7	2	2	12	3	9	0	24	6	25	16	67	2	8
Methionine ointment	12	3	6	3	2	12	4	6	2	24	7	29	12	50	5	21
Castilian malva infusion	12	2	8	2	2	12	4	7	1	24	6	25	15	63	3	12
Sulfanilamide powder	12	4	6	2	2	12	3	8	1	24	7	29	14	58	3	13
Sulfathiazole powder	12	3	6	3	2	12	2	7	3	24	5	21	13	54	6	25
Sulfathiazole ointment	12	3	7	2	2	12	4	6	2	24	7	29	13	54	4	17
Sulfadiazine spray	12	4	7	1	2	12	3	8	1	24	7	29	15	63	2	8
Scharlach R ointment	12	2	9	1	2	12	3	8	1	24	5	21	17	71	2	8
Tetrodine powder	12	6	4	2	2	12	5	6	1	24	11	45	10	43	3	12
Control	4	..	..	..	2	..	..	..	..	20						

intensity, a striking parallelism was observed in comparison with the previous clean and infected wound experiments. These data are summarized in Table VIII.

TABLE VII
COMPARATIVE RATE OF HEALING OF CLEAN AND INFECTED WOUNDS USING CHLOROPHYLL, VITAMIN OINTMENTS, SULFA COMPOUNDS AND TETRODINE

Test Agent	Accelerated		Unaffected		Delayed	
	Clean	Infected	Clean	Infected	Clean	Infected
Chlorophyll preparations	67.0	63.0	27.5	28.0	5.5	9.0
Vitamin ointments	18.4	19.4	71.6	70.8	6.0	9.7
Sulfa compounds	3.8	27.1	45.0	57.3	51.2	15.6
Tetrodine powder	5.0	45.0	60.0	43.0	35.0	12.0

As in the previous studies, the response in the rate of healing was found to be greater with the various chlorophyll preparations than with any of the other test agents, ranging from 62 to 83 per cent, with an average figure of 71 per cent. There seemed to be no striking relative differences in this rate between the larger (5.0 cm.), more severe burns and the smaller (2.5 cm.) lesions produced at a lower temperature, although obviously the larger lesions took somewhat longer to heal. In this series of animals the Bio-dyne did not seem to be particularly effective in hastening the repair process, although the resultant scar tissue formation was of excellent quality with but little contraction and deformity. The methionine and Scharlach R (amino-azotoluene azobetanaphthol) ointments and the Castilian malva wet dressings showed up a little better in this group of experiments with a slight acceleration of the healing process in from 40 to 50 per cent of the cases. It is our impression that these agents have more effect upon epithelization than upon the development of healthy granulation tissue as is the case with chlorophyll. Likewise, it should be noted that the actual amount of acceleration with these three agents seldom exceeded two to three days as compared with the 25 to 30 per cent obtained with chlorophyll. In the case of the vitamin preparations, a similar slight acceleration in the healing rate in some of the animals was observed, but this was counterbalanced by a like delay in others so that the net result was of no statistical significance. With the sulfa compounds a very definite delay in healing was found to be the rule, which was even more striking than that noted in the clean surgical wound series. Only in the presence of infection did the sulfa drugs exhibit their real effectiveness, permitting healing to proceed more promptly than in the control lesions through their bacteriostatic action.

TABLE VIII
HEALING OF EXPERIMENTALLY INDUCED, DRY HEAT, MILD, THIRD DEGREE BURNS IN DOGS

Test Agent	2.5 Cm.									5.0 Cm.										Summary							
			250°C.—20 Seconds				300°C.—30 Seconds				250°C.—20 Seconds				300°C.—30 Seconds					Healing							
			Healing				Healing				Healing				Healing												
	No. Animals	No. Lesions	Accelerated	Unaffected	Delayed	No. Lesions	Accelerated	Unaffected	Delayed	No. Lesions	Accelerated	Unaffected	Delayed	No. Lesions	Accelerated	Unaffected	Delayed	Total Number of Lesions Treated	Accelerated	Per Cent	Unaffected	Per Cent	Delayed	Per Cent
Chlorophyll—alkaline solution	4	8	6	2	0	4	2	2	0	8	5	2	1	4	2	2	0	24	15	62	8	33	1	5
Chlorophyll—lanoline ointment	4	4	2	1	1	8	5	3	0	4	3	1	0	8	6	1	1	24	16	67	6	25	2	8
Chlorophyll—jelly	4	4	3	1	0	4	4	0	0	8	6	2	0	8	7	1	0	24	20	83	4	17	0	0
Chlorophyll—wet dressing + ointment	4	8	6	2	0	8	6	1	1	4	2	1	1	4	2	2	0	24	16	67	6	25	2	8
Chlorophyll—wet dressing + jelly	4	6	5	1	0	6	4	2	0	6	4	2	0	6	5	1	0	24	18	75	6	25	0	0
Total chlorophyll	20	30	22	7	1	30	21	8	1	30	20	8	2	30	22	7	1	120	85	71	30	25	5	4
Bio-dyne ointment	4	6	1	4	1	6	2	4	0	6	1	5	0	6	1	3	2	24	5	21	16	67	3	12
Vitamin B ointment	2	3	1	2	0	3	0	2	1	3	0	3	0	3	1	2	0	12	2	17	9	75	1	8
Vitamin C ointment	2	3	2	1	0	3	1	1	1	3	0	2	1	3	1	2	0	12	4	33	6	50	2	17
Vitamin D ointment	2	3	1	1	1	3	2	1	0	3	1	2	0	3	0	2	1	12	4	33	6	50	2	17
Methionine ointment	2	3	2	1	0	3	1	2	0	3	1	1	1	3	2	1	0	12	6	50	5	42	1	8
Castilian malva infusion	2	3	1	2	0	3	1	1	1	3	2	1	0	3	1	2	0	12	5	42	6	50	1	8
Sulfanilamide powder	2	3	0	1	2	3	0	2	1	3	0	0	3	3	0	1	2	12	0	0	4	33	8	67
Sulfathiazole powder	2	3	0	2	1	3	0	2	1	3	0	1	2	3	0	2	1	12	0	0	7	58	5	42
Sulfathiazole ointment	2	3	0	2	1	3	0	1	2	3	1	2	0	3	0	1	2	12	1	8	6	50	5	42
Sulfadiazine spray	2	3	1	1	1	3	0	2	1	3	0	2	1	3	0	1	2	12	1	8	6	50	5	42
Scharlach R ointment	2	3	1	2	0	3	2	1	0	3	2	0	1	3	1	2	0	12	6	50	5	42	1	8
Tetrodine powder	2	3	1	2	0	3	1	1	1	3	0	2	1	3	1	2	0	12	3	25	7	58	2	17
Control	2	4	..	..	..	4	..	..	..	4	..	..	..	4	..	..	..	16						

TABLE IX
SUMMARY—ALL EXPERIMENTS

Test Agent	Total Lesions Tested	Accelerated		Unaffected		Delayed	
		No.	Per Cent	No.	Per Cent	No.	Per Cent
Chlorophyll preparations	448	304	67.9	120	26.7	24	5.4
Vitamin ointments	228	40	17.5	162	71.0	26	11.5
Sulfa compounds	304	31	10.1	144	47.4	129	42.5
Bio-dyne ointment	88	26	29.5	55	62.5	7	8.0
Methionine ointment	76	26	34.2	43	56.5	7	9.3
Castilian Malva infusion	76	27	35.5	43	56.5	6	8.0
Scharlach R ointment	76	23	30.2	43	56.5	10	13.3
Tetrodine powder	76	15	19.7	44	57.9	17	22.4
Totals	1,372	492	35.8	654	47.6	226	16.6

In Table IX will be found summarized the results of all three groups of experiments. It will be noted, that, including all three types of experimentally induced lesions in all four test animals, the rate of healing was accelerated appreciably in 67.9 per cent of the animals on which the various chlorophyll preparations were used. This percentage is approximately twice that obtained with any of the other preparations studied and five times as great as that recorded with the various sulfa compounds. In only 6 per cent of the animals on which chlorophyll was used was any delay in healing noted and in the majority of those cases there was secondary infection present not controlled by the drug.

COMMENTS

An attempt has been made in the foregoing experiments to evaluate the effect of topical application of various agents currently of interest in the treatment of wounds, of both traumatic and thermal origin. A standard procedure has been followed that the results might be critically compared. The method has the advantages of simplicity and uniformity which might make it useful to others working in this same field. By establishing such a standard technic, experimental studies from different laboratories could be more satisfactorily compared or contrasted, data could be easily duplicated for purposes of confirmation, and a universal understanding of each other's reports might hasten the solution of many acute problems in this field.

In these studies the objective has been strictly empirical and factual: to note the length of time required to heal a standard wound. No attempt has been made to theorize or to explain the manner of action of any of the agents under consideration. In earlier papers in this series, it has been shown that chlorophyll has a growth stimulating effect upon fibroblasts in tissue culture, and that it possesses certain bacteriostatic capacities. Its mode of action is still most obscure, but the practical application of these properties, which it has been shown to possess, to wound healing has been substantiated by the data presented in this report. We believe the use of chlorophyll should be extended widely in the clinical field possibly in conjunction with or subsequent to the more actively anti-bacterial agents such as the sulfa drugs. Its use in war injuries and burns

seems particularly indicated at this time in view of the results recorded here.

It has been of great interest to us to learn of the comparative ineffectiveness of any of the vitamins applied locally. While much of the recent work, such as that of Bartlett, Jones and Ryan[3] and Hunt,[17] has emphasized the value of an adequate vitamin C dietary intake in wound healing, its local application as demonstrated in these studies is practically negligible. Slightly more favorable results have been noted by Abramowitz[1] and Hardin[15] with the use of vitamin A and D ointments locally, but we have been unable to confirm this in these experiments. In the use of the sulfa drugs the resultant delay in wound healing is more than offset by their value in controlling infection. Our studies merely tend to confirm other, similar observations in this respect, notably those of Goldberger[13] and Bick.[4] In the clinical field the recent work of Reid,[22,23] Thompson and Ravdin,[26] Koster and Kasman,[18] Allen and Koch,[2] Whipple[28] and Elman[12] lay emphasis on the importance of correcting any existent hypoproteinemia which may exist. This aids in reducing the "lag" period by stimulating normal, healthy cell growth and providing the necessary adequate protein nutritional requirements.

Methionine was employed in this study with the idea of supplying the well established growth stimulating sulfhydryl radical. The results would seem to point to at least a partial utilization of this factor, although nothing very conclusive can be drawn as an inference with such a relatively small number of lesions. Castilian malva has been used in certain parts of the southwest and Mexico, more or less empirically, in various circulatory disturbances of the extremities accompanied by tissue breakdown with what have been claimed to be most encouraging results. It is now under laboratory and clinical investigation to learn something of its effects and pharmacologic action. In our studies, infusions, prepared from the crude, dried leaf have been used both in tissue culture and wound healing experiments. That the drug has some definite pharmacologic activity appears unquestionable, and further investigation into its behavior seems indicated.

In these wound healing experiments Bio-dyne ointment has likewise shown some slight suggestion of accelerating the reparative process. Its antiseptic component has been fairly effective in controlling infection, but again, any beneficial results noted have not been particularly striking. With Tetrodine, it is our impression we are dealing with an agent with all the antiseptic effects of tincture of iodine, but with very few if any of the objectionable features of tincture. It does not appear to delay healing appreciably because of its relatively minimal toxic effect upon tissues, and indeed, healing is definitely hastened in infected wounds as compared with the control animals by overcoming the infection. In this respect, it seems more effective than any of the sulfa compounds studied. Scharlach R ointment appears to be very ineffectual in accelerating the wound healing mechanism. Its much vaunted epithelial stimulating action seems practically negligible in our experience, and this impression has been recorded likewise recently by Boehringer and by Brush and Lam.

SUMMARY AND CONCLUSIONS

1. The effect upon the healing of 1,372 experimentally induced wounds and burns by the topical application of seventeen medicinal preparations is presented.

2. A control series of 878 similar lesions is included.

3. The agents tested include: Chlorophyll (aqueous soluble derivatives) in five vehicles, vitamin A, vitamin B complex, vitamin C ointments, bio-dyne ointment, methionine ointment, Castilian malva infusion, sulfanilamide powder, sulfathiazole powder and ointment, sulfadiazine spray, Scharlach R ointment, and tetrodine dusting powder.

4. Of all these agents, only the chlorophyll preparations consistently showed any statistically significant effect in accelerating the healing of both traumatic and thermal wounds.

5. Wound healing in 448 lesions in this group was accelerated by 24.9 per cent in time, in 304 or 67.9 per cent of the cases.

6. Vitamin B, C and D ointments showed no appreciable effect.

7. Bio-dyne and methionine ointments and Castilian malva wet dressings (infusion) caused a slight acceleration of healing in somewhat less than a third of the cases, but of less than 10 per cent in time.

8. The sulfa compounds caused definite retardation of the healing process except in the presence of active infection.

9. Scharlach R ointment was essentially inert, acting merely as a protective dressing similar to boric ointment or petrolatum gauze.

10. Tetrodine, an active, stable, aqueous soluble iodine preparation (with an iodine content of 4 per cent combined iodine and 2 per cent free iodine) reduces the healing time in about half the cases when infection is present. In the absence of infection some slight delay in healing is found presumably because of minor tissue irritation.

11. On the basis of these observations it is suggested that chlorophyll preparations should be used much more extensively in the treatment of wounds and burns.

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