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Book Review

Laboratory Instruction in Biochemistry, by ISRAEL S. KLEINER and LOUIS B. DOTTI. 188 pages. $7\frac{3}{4} \times 10\frac{1}{2}$ (loose leaf). 1940. St. Louis: C. V. Mosby Co. \$1.50.

This is a collection of laboratory experiments in elementary biochemistry on individual punched sheets which can be included in a laboratory notebook. The collection is intended for medical students and includes simple directions for elementary experiments in the biochemistry of carbohydrates, fats, proteins, milk, blood, urine, metabolism, food, etc. While intended primarily for medical students, it should also be of interest to pharmacy teachers and students.—A. G. D.

Further Observations on the Use of *Aloe vera* Leaf in the Treatment of Third Degree X-Ray Reactions*

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In 1940 one of us (T. D. R.) published a preliminary report on the use of fresh *Aloe vera* jell in the treatment of third degree x-ray reactions on white rats (1). Attention was called at that time to the modern use of this leaf in the treatment of such reactions in humans. It should also be pointed out that various species of *Aloe* have been employed for centuries to promote the healing of wounds and fire burns. Thus, Turner in 1568 (2) cited the use of "the herbe Aloe is to hele wounds," and Coxe in 1818 (3) referred to the use of powdered aloe "to check heamorrhagies in recent wounds." Various other references have from time to time mentioned aloe, either fresh or dry, as a healing agent. The present report deals with further observations on the use of the fresh jell, or pulp, of the leaf, as well as other portions of the leaf, in the treatment of experimentally produced third degree X-ray reactions on the skin of white rats.

The procedure followed in producing the X-ray reactions and the method of treatment in the present report are essentially the same as those described in the first report, with the following differences: rats were given a single dose of 4000 r instead of in divided doses since it was found that a single dose at this level was not toxic; ether was used for anesthesia in place of pentobarbital; control areas received no treatment of any

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kind; the duration of treatment was from two to four weeks.

The leaves used in the present report were obtained from the Jamaica gardens of S. B. Penick and Company.¹ They were sent in separate shipments of 50 to 155 each at intervals during the year 1940-1941 and were, with the exception of the last two shipments, in excellent condition when received. The following table summarizes the information concerning the various shipments.

Table I. *Aloe vera* Leaves Used

Shipment No.	Number of Leaves	Date Received	Condition on Arrival	Used in Treating Rat Group No.
2	55	8/9/40	Good	6
3	100	9/19/40	Good	7 and 8
4	105	11/8/40	Good	9
5	80	3/14/41	Good	10 and 11
6	155	3/31/41	Poor ^a	12, 13, 14 and 15
7	90	4/18/41	Poor ^a	12, 13, 14, 15 and 16

^a Many of the leaves of these shipments were completely decomposed and had to be discarded. The boxes in which they were shipped were stained a deep pink, indicating that much of the juice or latex from the rind had drained out enroute.

EXPERIMENTAL

Treatment with Fresh Pulp (Jell).—Three groups of rats were treated with this material. Group 6 was treated twice daily for four weeks while Groups 7 and 8 were treated twice daily for three weeks. The shorter period of treatment was resorted to because it was found that spontaneous healing affected the results after three weeks. The results are summarized in Table II.

Table II. Treatment with the Fresh Pulp

Rat Group No.	Improvement with Treatment			Improvement of Control Areas	
	Definite	Slight	No Difference	Definite	Slight
No. 6 14 rats	8	3	3	0	0
No. 7 16 rats	8	2	6	0	0
No. 8 14 rats	5	2	4	1	2
Totals	21	7	13	1	2

By combining the results in the first two columns it may be seen that 28 areas, or 63.8% of those treated, showed an increased rate of healing. This is 9 1/2 times as many as showed improvement in the control areas. A significant observation, made during this part of the study, was that those treated areas which showed an increased rate of healing usually did so within two weeks after treatment was started. If no beneficial results had been obtained within this period, continuation of the treatment seldom proved of benefit.

¹ Our thanks are due Mr. S. B. Penick for his kind cooperation in furnishing the leaves used in this study.

By combining the results obtained in the present study with those from the previous report (1) a complete summary of the treatment of a total of 72 rats with the fresh pulp may be shown as in Table III:

Table III. Treatment of 72 Rats with Fresh Pulp

No. of Rats Treated	Improvement with Treatment		No Difference	Improvement of Control Areas	
	Definite	Slight		Definite	Slight
72	31	11	21	2	7

From this it may be seen that a total of 42 areas, or 58.3% of those treated, showed an increased rate

of healing. This is 4 2/3 times as many as showed improvement in the control areas, and is a definite indication that a healing agent is present in the fresh pulp of *Aloe vera* leaf.

Treatment with the Partially Decomposed Pulp.—Some of the leaves from Shipment 4, after standing at room temperature for one month, were partially decomposed, while parts of them were completely spoiled. The latter areas were cut out and the remainder of the partially decomposed leaves stored at 5° C. After two months the pulp from these leaves had acquired a pink color and a characteristic fermented odor, and had lost most of its viscid, gummy consistency. Treatment of a group of 8 rats (Group 9) with this partially decomposed pulp resulted in 7 areas, or 87.5% of those treated, showing an increased rate of healing, while only one area, or 12.5%, showed no benefit. Moreover, all of the treated areas which responded did so within 12 days after treatment was started. Thus 3 areas were healed in 6 days, 1 area in 9 days, 2 areas in 11 days, and 1 area in 12 days. These results were better than those obtained with the fresh pulp and pointed to the conclusion, contrary to the statement of MacKee (4), that the pulp does not have to be fresh in order to be effective.

Treatment with the Fresh Rind.—The promising results obtained with both the fresh pulp and the partially decomposed pulp led us to suspect that the healing agent might be present originally in the rind and upon standing pass from the rind into the pulp. Accordingly, a group of 8 rats (Group 10) was treated with fresh rind prepared from Shipment 5 to study its effectiveness.

The rind was prepared by removing the pulp from the inside of the leaves by scraping, running the pulp-free rind through a meat grinder, and

thoroughly triturating the minced rind in a mortar, adding one ounce of distilled water to each four ounces of minced rind. This mixture was then applied in the form of a poultice to the areas which had been X-rayed in the usual manner.

All of the 8 areas, or 100%, thus treated were completely healed in 6 days, 5 areas having healed in 5 days. In order to obtain further data on the fresh rind, 4 of the control, or posterior, areas in this group were treated with the mixture, using the 4 remaining posterior areas as controls. Of these 4 posterior areas on which treatment was started 5 days later than usual, two areas were completely healed after 5 days' treatment and the other two were completely healed after 7 days of treatment.

Thus all of the 12 areas (8 anterior and 4 posterior), or 100%, treated with the fresh rind showed a definite increase in the rate of healing. These results, the best obtained with any portion of the leaf, seemed to indicate that the healing agent is present in higher concentration or in a more effective form in the fresh rind of the leaf.

Treatment with Aqueous Extracts of the Dried Rind from Shipment 6.—The positive results obtained with the fresh rind from Shipment 5 pointed to the fact that the healing agent was concentrated in the rind. If the fresh rind could be dried without loss of its effectiveness, then the dried rind could be made available for treatment of X-ray reactions in the form of an aqueous extract or poultice. Hence it seemed desirable to study the effect of drying on the rind and to see if the healing agent were water-soluble. Accordingly, the supply of leaves from Shipment 5 having been exhausted, two aqueous extracts were made from leaves of Shipment 6. The first was made by drying finely chopped rind in a vacuum desiccator for 72 hours, grinding the product to a fine powder, macerating the powder for 10 days with sufficient water to make a 10% mixture, and filtering. The second was made by drying finely chopped rind in an oven at 70° for 24 hours, powdering, macerating the powder with 9 times its weight of water at 70° for 3 hours, and filtering.

The filtrates in each case were used in the treatment of two groups of 8 rats each (Group 12) by saturating cotton packs and placing them on the irradiated areas. Neither of the extracts thus obtained produced an increase in the rate of healing. This would indicate that the healing agent, if present, was either insoluble in water or unstable to drying and extraction. However, these indications could not be conclusive until the fresh rind, from which the extracts were prepared, was shown to be either effective or ineffective as a healing agent.

Treatment with the Fresh Rind of Shipments 6 and 7.—In view of the disappointing results obtained in the preceding experiment with aqueous extracts of the dried rind from these shipments, it was considered desirable to test the effectiveness of the fresh rind itself from these shipments. The fresh rind was prepared, as previously described, by removing the pulp, mincing the rind, and making a poultice by

triturating it with distilled water. Two separate groups of 8 rats each (Groups 14 and 15) were treated with this material. Of these only 3 areas, or 18.7% of those treated, showed an increased rate of healing, the other treated areas showing no change.

These results explain the failure to obtain positive results with the aqueous extracts of the dried rind prepared from Shipments 6 and 7 in the preceding experiment and also seem to contradict directly the excellent results obtained with the fresh rind of Shipment 5. However, two possible explanations are offered for these negative results. The first is in the poor condition of the leaves of Shipments 6 and 7 when received. Most of the juice, or latex, had drained from the leaves during shipment and it is probable that the healing agent was lost in this way. The second, suggested by a producer of *Aloe vera* leaves (5), is that the healing agent may not be present in the leaves at all times but is found there only during certain seasons of the year.

Treatment with the Partially Decomposed Pulp from Shipment 7.—In order to determine whether the healing agent might have passed from the rind of these leaves into the pulp, a group of 8 rats (Group 16) was treated with the partially decomposed pulp from this shipment. Again, as was the case with the fresh rind and aqueous extracts of the dried rind from this shipment, disappointing results were obtained, only 2 areas, or 25% of those treated, showing an increase in the rate of healing.

Clinical Use of Fresh Rind from Shipments 6 and 7.—After the excellent results obtained from the experimental use of the fresh rind from Shipment 5, it was thought desirable to try the fresh rind clinically on a patient who had been suffering from an X-ray reaction of 7 months' duration and which had failed to respond to any treatment. Unfortunately, however, all of the leaves from Shipment 5 had been exhausted and only leaves from Shipments 6 and 7 were available. Twice daily treatments for one month with a poultice made by mincing the fresh rind with water failed to produce any response in the area involved. This negative result was in line with the same disappointing results obtained in the rat treatments, which were being run simultaneously with the clinical case, with leaf materials from these two shipments.

Treatment with Ointment of Dried Aloe.—In 1937 and 1939 Crewe (6, 7) reported on the beneficial results obtained by treating pruritus vulvae, ivy poisoning, and various types of fire burns with ointments of powdered aloe. These reports led us to try the effectiveness of an ointment of aloe on third degree X-ray reactions. An ointment of Curaçao aloe was made according to Crewe's directions consisting of powdered aloe, 2 drams, mineral oil, 2 drams, white petrolatum, 1 ounce. In a group of 7 rats receiving twice daily treatments for three weeks of this ointment none of the areas showed an increased rate of healing. These results showed that the particular sample of aloe used contained no healing agent for this type of reaction.

Treatment with Scarlet Red Ointment.—A 5% scarlet red ointment is sometimes used in the treatment of X-ray reactions on humans. In order to learn its effectiveness on the test animals, a 5% ointment of Biebrich's medicinal scarlet red in a petrolatum base was used in treating a group of 7 rats twice daily for 3 weeks. Of these none of the treated areas showed an increased rate of healing and in some the time of spontaneous healing appeared to have been prolonged.

Treatment with Urea Ointment.—Various reports (8, 9, 10, 11) in recent years have dealt with the effectiveness of urea in the healing of various types of wounds. In order to study its effectiveness in the treatment of third degree X-ray reactions, an ointment containing urea, 15 Gm., water, 10 cc., aquaphor, *q. s. ad* 100 Gm., was made and applied twice daily to a group of 8 rats. Of these only one area, or 12.5% of those treated, showed an increased rate of healing. These results indicate that urea is ineffective in such treatment and, furthermore, that urea is not the healing agent in the *Aloe vera* leaf.

SUMMARY AND DISCUSSION²

1. Further observations are reported on the use of *Aloe vera* leaf in the treatment of third degree X-ray reactions on white rats. Sufficient data have been obtained to show that treatment with the pulp of the leaf definitely increases the rate of healing of such experimentally produced reactions. Furthermore, contrary to previous views, our results indicate that the pulp does not have to be fresh in order to be effective as a healing agent. It should be noted that all of the reactions produced in this study are of the acute type and not of the chronic type which sometimes appear on humans many months after the original irradiation.

2. One source of error was noted during the study: The anterior areas of the rats were more resistant to irradiation than the posterior areas. The anterior areas were sometimes smaller than the posterior at the beginning of treatment; consequently, the former occasionally healed normally at a faster rate than the latter. This fact makes the observed results for increased rate of healing somewhat high for all groups of rats treated, but in the later parts of the study the error was overcome to a certain extent by treating alternate anterior and posterior areas.

² Our thanks are due Dr. E. A. Pohle, Professor of Radiology, Univ. of Wis., for his kind cooperation in this study.

3. Contradictory results were obtained with the fresh rind of the leaf, the rind from one shipment of leaves being more effective than the pulp in promoting healing, while the rind from two other shipments of leaves gave negative results. The different season of the year in which the latter shipments were collected, or, perhaps more likely, the poor condition of these leaves when received, is offered as a possible explanation for these negative results. It is believed, from our observations, that the healing agent of the leaf is concentrated in the rind. Further work is under way to clear up this question.

4. Results obtained with aloe ointment, scarlet red ointment and urea ointment show that none of these are effective in promoting healing of acute third degree X-ray reactions in the skin of white rats.

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CORRECTION

July, 1941, Issue, page 181.

Change heading of list of compounds in second column from "Substituted Acetylureas" to "Substituted Acetamides."