

A QUANTITATIVE ANALYSIS OF THE INFLUENCE OF THE
SIZE OF THE DEFECT ON WOUND HEALING
IN THE SKIN OF THE GUINEA PIG.

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The investigations of Loeb, Addison, and Spain have shown that normal as well as regenerative growth of the epidermis of various species of animals differs markedly, that there is a distinct relationship between the structures of the various skins and their proliferative energy, and that the structure of the normal skin and its behavior during regeneration are apparently determined by the proliferative energy of the cells.¹

We undertook to determine the influence of the size of the wound on the rate of wound healing in the different types of skin, and in this communication we deal with the results obtained in the guinea pig. In one set of animals a thin flap of skin measuring approximately 2 sq. mm. was removed from the ear of each animal; in another set flaps measuring 4 sq. mm. were removed. After 2, 5, 7, 9, 11, and 14 days pieces were cut out in each set for microscopic examination. Three to five pieces were examined at each period, in both the 2 sq. mm. and the 4 sq. mm. series. All the pieces were cut into several sections. The same measurements were made as in the previous investigations. The results are given in Tables I, II, III, and IV.²

¹Loeb, L., and Addison, W. H. F., *Arch. f. Entwcklungsmechn. d. Organ.*, 1911, xxxii, 44. Addison, W. H. F., and Loeb, L., *Arch. f. Entwcklungsmechn. d. Organ.*, 1913, xxxvii, 635. Spain, K. C., *Jour. Exper. Med.*, 1915, xxi, 193.

²The microscopic measurements were carried out by Dr. Spain.

TABLE I.

Four Millimeter Series.

Day.	No. of piece.	Length of tongue.	Length of defect.	Length of tongue and defect.	No. of living cell rows and width of stratum germinativum.			
					Tip of tongue.	Insertion of tongue.	Old epithelium.	
2	1 2 3	<i>mm.</i> 0.498 0.454 0.526 0.619 0.462 0.511	<i>mm.</i> 3.0 2.813 3.113	<i>mm.</i> 3.952 3.954 4.086 3.113	1-3	4-5	4	
					(14.6 μ)	(47.1-53.8 μ)	(41.3 μ)	
					1-3	4-5	4	
					(15.3 μ)	(49.53-57.6 μ)	(45.3 μ)	
3½	1	0.0823 0.0965	0.894	2.0	3.788	1-3	5-7	5
						(19.2 μ)	(55.2-67.4 μ)	(56.9 μ)
5	1 2 3	Closed			2.137 2.167 2.772	Middle of wound.		
						7	7-11	7
						(76.3 μ)	(69.2-117.3 μ)	(64.7 μ)
						6	7-11	7
7	1 2 3	"			1.567 1.662 1.852	6	7-11	7
						(73.9 μ)	(88.4-119.3 μ)	(68.7 μ)
						6	7-11	7
						(69.2 μ)	(84.0-126.9 μ)	(65.3 μ)
9	1 2 3	"			1.514 1.715 1.562	7	5-7	5
						(70.3 μ)	(57.2-83.4 μ)	(53.8 μ)
						6	5-7	5
						(62.8 μ)	(62.6-93.3 μ)	(53.4 μ)
11	1 2 3	"			1.514 1.715 1.562	6	5-7	5
						(61.7 μ)	(61.5-88.1 μ)	(56.1 μ)
						5	5-7	5
						(58.0 μ)	(59.2-79.6 μ)	(46.2)
14	1 2 3	"			1.514 1.715 1.562	5	5-7	5
						(55.6 μ)	(48.5-65.7 μ)	(48.4 μ)
						4	5-7	4
						(41.1 μ)	(52.6-64.4 μ)	(39.6 μ)
11	1 2 3	"			1.372 1.309 1.330	5	5-7	5
						(58.0 μ)	(59.2-79.6 μ)	(46.2)
						4	5-7	4
						(41.1 μ)	(52.6-64.4 μ)	(39.6 μ)
11	1 2 3	"			1.372 1.309 1.330	3	5-7	4
						(32.5 μ)	(42.3-54.5 μ)	(37.3 μ)
						3	5-7	4
						(29.6 μ)	(49.9-67.6 μ)	(45.7 μ)
14	1 2 3	"			1.228 1.086 1.079	3	5-7	4
						(26.9 μ)	(53.4-60.7 μ)	(39.9 μ)
						3	5-7	4
						(30.7 μ)	(48.6-57.4 μ)	(34.6 μ)
14	3	"			1.079	3	5-7	4
						(28.5 μ)	(51.9-55.3 μ)	(43.0 μ)

TABLE II.
Four Millimeter Series.

Day	No. of piece.	Size of cell.	Size of nucleus.	No. of mitoses in one area 1 mm. long x 50 μ wide.		
				New epithelium.	Old epithelium.	
2	1	μ 15.3 x 9.6	μ 11.6 x 7.6	0	29	
	2			1 } $\frac{1}{3}$		28
	3			0		32
3 $\frac{1}{2}$	1	16.3 x 9.7	11.8 x 7.5	38	30	
	2					
	3					
5	1	12.1 x 9.7	9.1 x 6.5	4	15	
	2			7 } $6\frac{2}{3}$		12
	3			8		14
7	1	12.6 x 9.8	9.3 x 6.9	4	15	
	2			4 } 5		16
	3			7		15
9	1	11.9 x 9.6	9.2 x 6.9	4	14	
	2			5 } $4\frac{2}{3}$		13
	3			5		15
11	1	11.8 x 9.7	8.7 x 6.4	4	13	
	2			3 } $3\frac{1}{3}$		12
	3			3		11
14	1	11.2 x 9.7	8.9 x 6.1	4	13	
	2			3 } $3\frac{1}{3}$		12
	3			3		13

Changes in the Size of the Wound and Tongue.

The various headings under which the data are arranged in these tables are the same as in the tables in our preceding communications.³ Although the figures of only three pieces are given in the tables, in the majority of cases additional pieces were examined, and on the whole the results were concordant. In the third column of Tables I and III we find the figures for the length of the tongue in the various periods. Two days after making the wound a tongue

³ Addison, W. H. F., and Loeb, L., *Arch. f. Entwicklungsmechn. d. Organ.*, 1913, xxxvii, 635. Spain, K. C., *Jour. Exper. Med.*, 1915, xxi, 193.

TABLE III.
Two Millimeter Series.

Day	No. of piece.	Length of tongue. <i>mm.</i>	Length of defect. <i>mm.</i>	Length of tongue and defect. <i>mm.</i>	No. of rows of living cells and width of stratum germinativum.			
					Tip of tongue.	Insertion of tongue.	Old epithelium.	
2	1	0.30	1.504	2.03	1-2	3-4	3-4	
		0.23			(13.4 μ)	(30.6-43.8 μ)	(34.7 μ)	
	0.38	1.426		1.956	1-2	3-4	3-4	
	0.15				(12.6 μ)	(28.7-45.6 μ)	(37.8 μ)	
2	0.25	1.68	2.23	1-2	3-4	3-4		
3	0.28			(11.5 μ)	(38.4-41.9 μ)	(33.6 μ)		
5	1	0.538	0.83	2.15	3-4	7-11	7-9	
		0.682			(38.9 μ)	(91.3-115.3 μ)	(69.2 μ)	
		0.513			3-4	7-11	7-9	
	0.787	0.984		1.98	(46.9 μ)	(90.9-109.6 μ)	(66.3 μ)	
	0.692				3-4	7-11	7-9	
3	0.511	0.782	(42.3 μ)	(88.8-111.6 μ)	(71.6 μ)			
7	1	1.076	0.007	1.852	5-6	7-11	6-7	
		0.769			(63.3 μ)	(78.0-123.07 μ)	(68.5 μ)	
	2	Closed		1.662	Middle of wound.	5-6	7-11	5-6
					(64.2 μ)	(78.6-84.7 μ)	(58.8 μ)	
3	"	1.568	5-6	7-11	5-6			
			(62.7 μ)	(72.6-89.6 μ)	(61.5 μ)			
9	1	"		1.461	3-4	7-9	4-5	
	2	"		1.388	(46.1 μ)	(69.3-78.4 μ)	(48.4 μ)	
	3	"		1.569	3-4	7-9	4-5	
					(52.3 μ)	(72.3-84.2 μ)	(49.9 μ)	
					(48.2 μ)	(60.8-80.7 μ)	(52.8 μ)	
11	1	"		1.204	3-4	5-7	4-5	
	2	"		1.406	(48.2 μ)	(50.8-69.2 μ)	(44.2 μ)	
	3	"		1.284	3-4	5-7	4-5	
					(41.5 μ)	(50.2-64.7 μ)	(41.2 μ)	
					3-5	5-6	4-5	
					(53.8 μ)	(49.7-67.2 μ)	(43.1 μ)	
14	1	"		1.208	3-4	5-7	3-4	
	2	"		1.197	(37.9 μ)	(46.1-57.6 μ)	(36.4 μ)	
	3	"		1.228	3-4	5-7	3-4	
					(38.4 μ)	(42.3-61.5 μ)	(44.2 μ)	
					3-4	5-7	3-4	
					(42.3 μ)	(51.9-63.8 μ)	(35.3 μ)	

TABLE IV.
Two Millimeter Series.

Day.	No. of pieces.	Size of cell.	Size of nucleus.	No. of mitoses in one area 1 mm. long x 50 μ wide.	
				New epithelium.	Old epithelium.
2	1	"	"	1	44
	2	13.4 x 8.9	9.2 x 7.3	2 } 1 $\frac{1}{2}$	45 } 44
	3			1	43
5	1			28	26
	2	14.6 x 9.9	10.3 x 7.9	26 } 26	23 } 24
	3			24	23
7	1	Open		Open 72	26
	2	14.6 x 9.7	9.8 x 7.6		
	3	Closed 12.1 x 8.6	9.5 x 7.5	Closed 42	20
9	1			27	20
	2	13.4 x 8.8	9.5 x 7.2	22 } 22	16 } 17 $\frac{1}{2}$
	3			17	17
11	1			15	14
	2	11.7 x 7.5	9.1 x 6.6	14 } 15 $\frac{1}{2}$	15 } 13 $\frac{1}{2}$
	3			17	12
14	1			11	13
	2	11.2 x 7.3	9.3 x 6.7	14 } 12 $\frac{1}{2}$	14 } 14 $\frac{1}{2}$
	3			12	16

of regenerating epidermis measures 0.265 mm. in the 2 mm. series, and almost twice as much in the 4 mm. series. After three and one-half days the tongue has increased considerably and it is closed after five days in the 4 mm. series. In the 2 mm. series the length of the tongue has enlarged considerably at this period. It is more than double as long as two days after the operation, but the wound is not yet closed. In two pieces the wound was closed after seven days, in one it was still open in the 2 mm. series. After nine days the wound is closed in all the series. The figures in the 3 mm. series, which Addison and Loeb communicated previously,⁴ stand generally

⁴ Addison, W. H. F., and Loeb, L., *Arch. f. Entwicklungsmechn. d. Organ.*, 1913, xxxvii, 635.

between the figures in the 2 and 4 mm. series. Slight variations which occur are probably due to the fact that in the previous work the exact size of the wound had as yet not been considered as of very great importance and there may therefore have been slight variations in the size of the original wound in the 3 mm. series. We may conclude from these figures that the larger the wound the more rapidly the tongue enlarges and the earlier the closure of the wound takes place, within the variations in size of the wound chosen in our experiments. This difference in the energy of outgrowth of the epithelial tissue is already noticeable within the first two days. Again, from the second to the fifth day the increase in size of the tongue over the size two days after the operation is absolutely greater in the 4 mm. than in the 2 mm. series.

A study of the changes in the size of the defects agrees with the changes in the size of the tongues (Column 4, Tables I and III). Two days after the operation the defect has decreased 1.025 mm. in the 4 mm. series and a little less than half as much (0.461 mm.) in the 2 mm. series. From two days to three and one-half days a further decrease of 0.81 mm. in the size of the defect has taken place in the 4 mm. series. The rate of the decrease is approximately the same as in the preceding period in the 4 mm. series. Sometimes between three and one-half and five days the defect disappears in the 4 mm. series. In the 2 mm. series it decreases 0.639 mm. from the second to the fifth day. Again the defect decreases from the second to the fifth day more in the 4 mm. than in the 2 mm. series. We find, therefore, that the decrease in the size of the defect is in the 4 mm. series very much more rapid than in the 2 mm. series. The figures for the 3 mm. wounds stand between those for 2 and 4 mm.

Contraction of Wound.—In the fifth column of Tables I and III we find the figures for the length of tongues plus defect or of the length of the former defect, after it has been covered by the tongues. The figures in this column after the closure of the wound and the difference between the decrease in the defect and the increase in the tongues in the different periods give an indication of the contraction which takes place in the area of the wound simultaneously with the outgrowth of the tongues.

If we consider first the 4 mm. series, we find no noticeable con-

traction before the end of the second day; between the second and third and a half day there is possibly a very slight contraction. There is a noticeable contraction beginning before the end of the fifth day. A decided contraction, therefore, takes place at the time of the closure of the wound and shortly preceding this period. Between the fifth and seventh day a further decided contraction takes place. Between the seventh and fourteenth day the contraction continues, but at a generally diminishing rate.

In the 2 mm. series the contraction begins much later than in the 4 mm. series, and again at the period of closure and the time preceding it; *viz.*, between the fifth and seventh days. During this period the contraction is quite noticeable. From the seventh to the fourteenth day the contraction continues here with gradually decreasing intensity. In the 4 mm., as well as in the 2 mm. series, the contraction is therefore most marked in the beginning and gradually decreases. At the time of closure the contraction amounts to about 0.4 to 0.5 mm. in both series. Between the ninth and eleventh days the contraction is in both cases somewhat greater than 0.2 mm. Fourteen days after the operation the area of the healed wound has shrunk in both series to about the same area. Therefore, the contraction that takes place in the 4 mm. series is absolutely much greater than that in the 2 mm. series. The amount of contraction in the two series differs, therefore, in the period preceding the seventh day. Between the seventh and fourteenth days the contraction is absolutely approximately the same in both series. The contraction is therefore greater in larger wounds. The approximate contraction in the two series is as follows (Table V).

TABLE V.

Days.	4 mm. series. <i>mm.</i>	2 mm. series. <i>mm.</i>
0 to 2		
2 to 3½	0.05	
3½ to 5	0.4	
5 to 7	0.68	0.5
7 to 9	0.09	0.22
9 to 11	0.22	0.23
11 to 14	0.14	0.04
Total	1.58	0.99

These figures show that both outgrowing of the tongues and contraction are concerned in the closing of the defect and that both are greater in the larger wound.

Mitoses.—The number of mitoses in the 4 mm. series is given in Table II, and in the 2 mm. series in Table IV. We distinguish between the mitoses in the tongue (here the number of mitoses in one of the tongues over an area $50\ \mu$ wide is counted) and in the old epithelium (the number of mitoses in the old epithelium is determined over an area extending 1 mm. from the insertion of the tongue into the old epithelium and $50\ \mu$ wide). After the closure of the wound the mitoses in half the epithelium covering the defect are counted.

At two days we find in the 4 mm. series in the tongue $\frac{1}{3}$ of a mitosis in one unit area, in the old epithelium 30 mitoses in a unit area; in the 2 mm. series there are $1\frac{1}{3}$ mitoses in one unit area in the tongue, and 44 mitoses in the old epithelium. As we stated in an earlier communication, at first there are almost no mitoses in the tongue and many mitoses in the old epithelium. In the animals with the smaller wounds there are more mitoses in the old epithelium, although the tongues are greater in the animals with the larger wounds. We may therefore conclude that the tongue formation is not directly dependent on the mitoses but on the migration of the cells. We notice furthermore that also in the tongue the mitoses are more frequent in the smaller wounds. Within a very short time the mitoses increase rapidly in the tongue, and at three and one-half days the mitoses are more frequent in the tongue than in the old epithelium in the 4 mm. series. In the old epithelium the number remains at three and one-half days about the same as at two days.

At five days the wound is closed in the 4 mm. series. We find therefore a sudden decline in the number of mitoses in both former tongue and old epithelium, but the decrease is greater in the tongue, where the pressure of cell layers which meet from opposite directions upon each other is greatest. In the 2 mm. series where the wounds are not yet closed the mitoses in the tongues increase markedly, just as the mitoses did in the tongues in the 4 mm. series at three and one-half days. In the old epithelium, however, no further increase, but a certain decrease takes place, which latter is, however, probably

not very significant and may perhaps be due to a relative diminution in the pull from the tongue.

At seven days the number of mitoses in the former tongue and old epithelium is similar to the number at five days in the 4 mm. series. In the 2 mm. series we must distinguish between those pieces in which a closure of the wound has not yet taken place, and those in which the wound has just closed. In the former the number of mitoses in the tongue increases very much (72), while in the old epithelium the number of mitoses has hardly undergone a change. In the latter directly after the closure a rapid decline in the number of mitoses in the tongues has set in; in these pieces the number of mitoses is about 50 per cent less than in the pieces in which the wound is not yet closed, while in the old epithelium only a relatively much smaller diminution has taken place after the closure (20 mitoses). In a similar way we found in the 3 mm. series a marked rise in the number of mitoses in the tongue and no increase, or rather a slight decline, in the old epithelium just before the time of closure of the wound. In the 4 mm. series the fall in the number of mitoses in the old epithelium directly after the closure of the wound is greater than in the 3 and 2 mm. series. In the latter series in the old epithelium a slight fall had already taken place before the closure of the wound.

At nine days the number of mitoses in the former tongue and old epithelium is about the same as at seven days; in the 4 mm. series there is at the most a very slight decrease. In the 2 mm. series there is a marked decrease in the number of mitoses in the old tongue and a slight decrease in the old epithelium. At both places, however, the number of mitoses is absolutely greater than after the closure of the wound in the 4 mm. series. In all three series the decrease in the number of mitoses after the closure of the wound is greatest in the tongue, while the number of mitoses in the old epithelium is subject to much less marked variations. We see that absolutely the number of mitoses remains higher in the 2 mm. than in the 4 mm. series. Thus the figures for the mitoses at nine days in the former tongues are $4\frac{2}{3}$ and 22 mitoses, and for the old epithelium 14 and $17\frac{2}{3}$ mitoses in the 4 mm. and 2 mm., respectively.

At eleven days there is a further slight decrease in the number of

mitoses in all three series in both the former tongues and the old epithelium; absolutely the decrease is the smaller, the greater the number of mitoses is at nine days.

At fourteen days only at certain places a further very insignificant decrease takes place, while at other places the figures remain almost unchanged. At this time the number of mitoses is approximately the same in all three series, with the exception of the region of the former defect in the 4 mm. series where the number of mitoses is less than elsewhere. The number of mitoses is now approximately the same as in the normal skin. We may conclude that the differences in the rapidity in the outgrowth of the tongues and the resulting differences in the time of closure are mainly responsible for the differences in the variations in the mitoses in the different series. Closure of the wounds is followed by a sudden decline in the number of mitoses, especially in the area of the defect. Therefore the number of mitoses decreases earliest in the 4 mm. series, and absolutely we find there the smallest number of mitoses during the period over which our observations extended. We find therefore a greater number of mitoses in the smaller wound. Of course we have to take into consideration the fact that our observations extend only over an area of the old epithelium 1 mm. long. It is possible that in the 4 mm. series the increase in mitoses extends over a longer area than in the 2 mm. series.

TABLE VI.

No. of Mitoses in the Tongue or in the Epithelium Covering the Former Defect in an Area 1 Mm. Long x 50 μ Wide.

Days.		
2	4 mm. series.	$\frac{2}{3}$
	2 " "	5
3 $\frac{1}{2}$	4 " "	42
5	4 " "	5.1
	2 " "	40
7	4 " "	6
	2 " "	88
9	4 " "	6
	2 " "	30
11	4 " "	4.7
	2 " "	25
14	4 " "	4.8
	2 " "	21

We have so far considered the number of mitoses in the tongues and in the epithelium covering the former defect independently of the size of the epithelial area. If we reduce all the figures to an epithelial area 1 mm. long, in the same way that we did in the case of the old epithelium, the character of the curve is not changed considerably, but we find in the 2 mm. series the number of mitoses in the region of the old defect exceeding those in the old epithelium and remaining above the normal number for a longer period of time than the figures given above indicate (Table VI).

Changes in the Size of Cell and Nucleus.

The size of the cell and nucleus were measured at the point of insertion of the tongue. Two diameters of cell and of nucleus in the 4 and 2 mm. series are given in Tables II and IV (Columns 3 and 4). If we consider only two diameters, the changes in cell and nucleus are represented in Table VII.

TABLE VII.

Days.	4 mm. series.		2 mm. series.	
	Cell.	Nucleus.	Cell.	Nucleus.
Normal cell.....	75	49	75	49
2	147	88	119	67
3 $\frac{1}{2}$	158	88		
5	117	59	145	81
7	124	64	142	75 (open).
			104	71 (closed).
9	114	64	118	68
11	115	56	88	60
14	109	54	81	62

We see that soon after the making of the wound the size of the cell and nucleus increase up to a maximum which is absolutely apparently only a little higher in the 4 mm. than in the 2 mm. series, which, however, is reached much earlier in the 4 mm. than in the 2 mm. series. The maximum is reached just before the closure of the wound in both series. After the closure a sudden decrease in the size of the cells takes place, and from that time on the size remains almost unchanged between the fifth and eleventh day in the 4 mm. series. At fourteen days a very slight decrease is observed, but the

size is still greater than in the normal epidermis. In the 2 mm. series a sudden decline takes place at the seventh day or soon afterwards, and from that time on the size of the cells continues to get smaller at a more rapid rate than in the 4 mm. series. But here also the cell size is still slightly above normal fourteen days after the making of the wound. The changes in the size of the nucleus follow a similar curve; but here the variations are smaller, and the deviations from the normal size not so great as in the case of the whole cell. On the whole, the curves indicating variations in cell and nuclear size, on the one hand, and in the number of mitoses run parallel to each other in the 4 mm. as well as in the 2 mm. series. But the size of cell and nucleus seems to return less quickly to the normal condition than the number of mitoses. Furthermore, the cell size increases more in the 4 mm. series than in the 2 mm. series, while the number of mitoses was more increased in the 2 mm. series.

Number of Rows of Living Cells and Width of Stratum Germinativum.

The last three columns in Tables I and III give the number of rows of living cells and the width of the stratum germinativum, at the tip of the tongue, insertion of tongue, and in the old epithelium near the insertion of the tongue. These figures are not so definite as those of the size of the cells, and in case there is an apparent contradiction between the two sets of figures, the figures for the size of the cells are to be preferred.

We see that at two days the number of cell rows is everywhere greater in the 4 mm. series than in the 2 mm. series, and the thickness of the stratum germinativum is also greater in the 4 mm. series. At three and one-half days the number of cell rows and the thickness of the stratum germinativum increase slightly. At five days the number of cell rows and the thickness of the living epithelium are still greater in both the 2 mm. and 4 mm. series; and the figures are similar in the 2 and 4 mm. series at the place of insertion of the tongues and in the old epithelium. The main difference exists in the epithelium covering the defect. In the 4 mm. series the two tongues have met at that time, exert a pressure on each other, and

we find, therefore, a very much thicker layer and more cell rows in the 4 mm. than in the 2 mm. series at this point. At seven days the figures are very similar to those at five days over the former defect in the 4 mm. series. From then until the fourteenth day a gradual decrease takes place in the thickness of the living epithelium as well as in the number of cell rows in this area.

In the 2 mm. series, on the other hand, we find the maximum in the number of cell rows and thickness of the living epithelium reached at the seventh day, again at the time of the closure of the wound. There the maximum is not so great as in the 4 mm. series. The push with which the two tongues coming from opposite sides meet each other is evidently greater in the 4 mm. series than in the 2 mm. series, and this push of the opposing tongues leads to the increase in the number of living cell rows and thickness of the epithelium in this area. Afterwards the epithelium evidently recedes again somewhat or is cast off. Between the eleventh and fourteenth day the decrease is greater in the 4 mm. series than in the 2 mm. series.

At the same time that the maximum is reached over the former defect, directly after the time of closure, a maximum is also reached at the point of insertion of the tongue and in the old epithelium in the 4 mm. series, while in the 2 mm. series the maximum at these two places is reached before the closure of the wound at the fifth day; from the direction of the old epithelium the push towards the wound extends into the tongue which leads to the subsequent closing. The maximum figures are very similar in the 2 and 4 mm. series. While, however, at the seventh day there is already a distinct diminution in the 4 mm. series, the decrease is less at seven days in the 2 mm. series at these two places; this is in accordance with the fact that at seven days the closure takes place in the 2 mm. series, while at that period the wound has already been closed for some time in the 4 mm. series. Afterwards in both series a gradual decrease takes place at these two areas. At the point of insertion the number of cell rows and the thickness of the epithelium are greater than in the old epithelium in both series, throughout the time of observation. From the eleventh to the fourteenth day the figures at these two places are very similar in the 4 mm. series to those in the 2 mm.

series. We may conclude that in both the 4 and in the 2 mm. series a push of the epithelium takes place in the direction towards the wound, and movements of the epithelium occur not only over the defect, but also in the neighboring epithelium, and that these movements may perhaps proceed in rows from the old epithelium towards the wound, so that a maximum is reached in the old epithelium at a slightly earlier period than over the defect. It thus appears that the primary process in the wound healing consists in movements of the epidermis towards the wound, that these movements are carried out with greater energy if the wound measures 4 mm. than if it measures 2 mm., that the pull of the epithelium calls forth mitotic cell division, and that pressure exerted by epithelial cells upon each other leads to a rapid diminution in the mitotic proliferation.

SUMMARY.

1. The larger the wound, the more rapidly the tongue enlarges and the earlier the closure of the wound takes place. Larger wounds heal, therefore, more quickly than smaller wounds within the variations in the size of the wound chosen in our experiments.

2. Both outgrowing of the tongues and contraction of the wound are concerned in the closing of the wound. A marked contraction sets in in the period preceding the closing of the wound and continues over a longer period with gradually diminishing intensity. The contraction, therefore, sets in earlier in the larger wounds. The contraction is also absolutely greater in the larger wound.

3. During wound healing the mitoses increase first markedly in the old epithelium and only very few mitoses can be found in the outgrowing epithelium during the first two days. Very soon the mitotic proliferation extends to the tongue and the number of proliferating cells may here become greater than in the old epithelium. With the closure of the wound a sudden fall in the number of mitoses takes place in both series. This fall is greatest in the tongue. Throughout the time of observation the number of mitoses is greater in the smaller wound. The fall in the number of mitoses directly after the closure of the wound is more sudden in the 4 mm. than in the 2 mm. series. It is, however, possible that the increase of mitoses extends over a larger area in the 4 mm. series than in the 2 mm. series.

4. It is probable that the difference in the rapidity in the outgrowth of the epidermal tongues and the resulting difference in the time of closure are mainly responsible for the difference in the variations in mitoses in the larger and smaller wounds. The longer the period of time over which the pull of the epithelium extends, the greater is the number of mitoses in this area. Therefore it is greater in the 2 mm. series. Closure of the wound is followed by a sudden decline in the number of mitoses especially in the area of the defect. Therefore the number of mitoses decreases earlier in the 4 mm. series, and we find here the smallest number of mitoses during the whole period of our observation.

5. The size of the epithelial cell and nucleus increases soon after the making of the wound. A maximum is reached in both the larger and smaller wound in the period just preceding the closure of the wound; this maximum is therefore reached earlier in the larger wound. Absolutely the maximal size reached in both kinds of wounds is approximately the same or only a little higher in the larger wounds. After the closure of the wound a sudden decline in the cell size takes place in the larger as well as in the smaller wounds. Then a more gradual decline sets in. Fourteen days after the operation the cells are still larger than in the normal skin. The variations in the size of the nucleus are similar to those in the whole cell, but less marked. The curves of variations in cell and nuclear size follow in both the larger and smaller wounds a curve similar to the variations in the number of mitoses. But the cell size returns more slowly to the normal condition than the number of mitoses.

6. The closure of the wound causes an increase in the number of epithelial rows over the defect. This increase is therefore reached at an earlier period in the larger wound. The increase is greater in the larger wound owing to the greater pressure which the two opposing cell layers exert upon each other in the larger wound. In the old epithelium the maximum in the number of cell rows is apparently reached slightly before the closure of the wounds. It seems that the epithelial movements leading to the closure of the wound start in the old epithelium and extend wave-like towards the wound.

7. It thus appears that the primary process in the wound healing consists in movements of the epidermis towards the wound, that

these movements are carried out with greater energy in the case of the larger wounds, that the pull of the epithelium calls forth mitotic cell division, and that pressure exerted by epithelial cells upon each other leads to a rapid diminution in the mitotic proliferation.