THE AGE FACTOR IN THE VELOCITY OF THE GROWTH OF FIBROBLASTS IN THE HEALING WOUND*

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Wounds heal with greater rapidity in the young than in adults. Du Noüy has (1) demonstrated this experimentally. He found that in epithelial wounds of equal size the rate of healing varied in inverse ratio to age; *i.e.*, the more rapid rate was in the young and aging constantly diminished the velocity. An increased rate of cellular proliferation has generally been accepted as the explanation for the greater rapidity of healing, particularly as growth is characteristic of this period of life. It was suggested, however, in a previous paper by one of us that this increased rate of healing might be attributable to another cause,—a diminution in the amount of retardation entering into the process. This retarding factor is apparently what limits the mass of tissue regenerated to a volume just sufficient to replace the damaged tissue (3), and evidently it plays a large rôle in the healing for the accelerated phase with its increasing daily increments of strength is very brief, as contrasted to the longer retarded phase, with its daily increments of strength continually decreasing.

To determine whether the rate of fibroplasia is actually greater in the wounds of the young and what the relation is between the accelerated and retarded phases of repair is the object of this investigation. A velocity curve of healing for wounds in the stomach of young rats will be derived by the method previously employed for the adult rats and the two curves will then be compared. A comparison will also be made with another velocity curve of healing in which there was a definitely increased rate of fibroplasia (3). This curve was obtained by feeding animals on a high protein diet. Clark was the first investi-

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gator to demonstrate that food could influence the rate of healing (2). He found a shortening of the latent period before healing began in wounds of the skin when the dogs were fed a high protein diet, and the entire length of healing time was correspondingly diminished. A high fat diet had the opposite effect—prolongation of the latent period and an extended healing time.

EXPERIMENTATION

Healthy young rats of our own stock, weighing from 58 to 85 gm. and varying in age from 35 to 48 days, were used. They were kept in separate cages and fed on the standard diet previously employed in the adult rats (Table I). Until the time of operation they had had normal growth curves.

TABLE I

Standard Balanced Diet (Smith and Moise)

Composition	Calories per kilo of food	Apportionment of total calories
per cent		*per cent*
Casein..... 18	738	Protein..... 13.8
Starch..... 51	2,091	Carbohydrate..... 39.2
Crisco..... 22	2,046	Fat..... 47.0
Cod liver oil..... 5	465	
Salts (Osborne and Mendel mixture)..... 4		
Lettuce twice a wk. 70 mg. of yeast daily	5,340	

After being fasted for 6 hours, an incision of approximately 1 cm. in length was made in the cardiac portion of the stomach and sutured in two layers with No. 000 plain catgut. Under these circumstances this size catgut loses its tensile strength in 48 hours (5). The same technique of suturing was always employed, the mucosa being first approximated and then the serosa with slight inversion. The abdominal wall was closed with silk. After this procedure the rats were immediately returned to their cages and the diet. At definite intervals after the operation, five rats were killed and the strength of the wounds determined. Animals were discarded with infected wounds or with pathology in other organs. To test the strength of the wound, the stomach was excised, the esophagus tied off, and a cannula inserted in the pylorus. Air was then admitted at a constant rate of speed from a constant head of pressure. The force required to rupture the wound was registered on a rotating drum by means of a mercury manometer

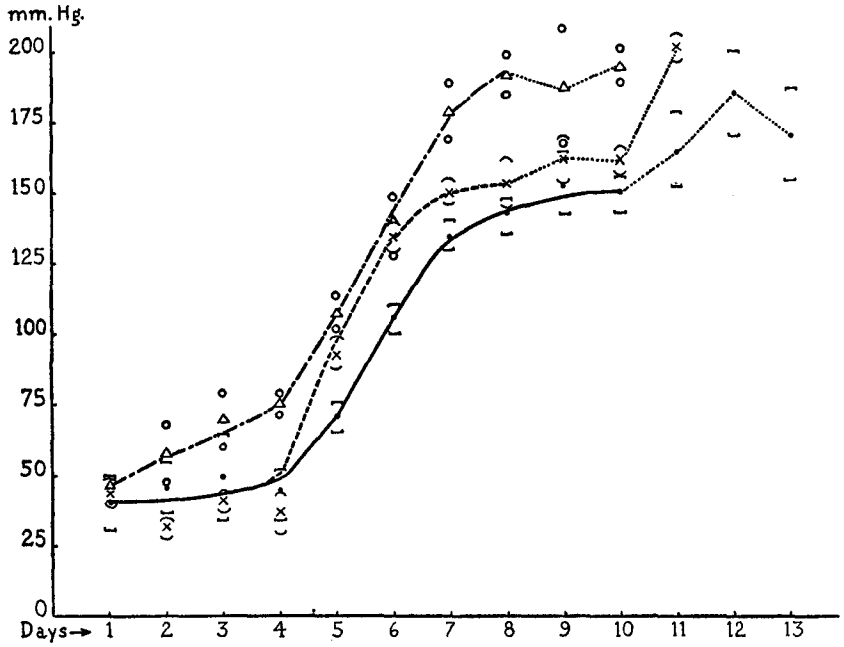
equipped with a writing point. The details of this technique have been described previously. To test the strength of the holding power of the sutures alone, wounds were repaired and tested in five dead animals.

TABLE II

P. O.	Young rats (standard diet)			Adult rats (standard diet)			Adult rats (protein diet)		
	Average strength	Standard deviation of mean	Nature of break	Average strength	Standard deviation of mean	Nature of break	Average strength	Standard deviation of mean	Nature of break
days									
1	60	±10.5	In incision	41.5	±9.0	In incision	45.4	±4.0	In incision
2	58	±10.5	" "	47.5	±9.0	" "	33.6	±4.0	" "
3	70	±9.0	" "	49.9	±15.0	" "	42.0	±3.0	" "
4	76	±2.0	" "	45.8	±8.0	" "	38.6	±7.0	" "
5	102	±6.0	" "	71.0	±5.0	" "	94.0	±5.4	" "
6	140	±9.0	" "	107.0	±6.0	" "	135.0	±5.0	" "
7	178	±10.0	" "	135.0	±5.4	" "	150.0	±5.3	" "
8	192	±7.0	" "	143.0	±6.8	" "	154.0	±9.0	2 elsewhere 1 alongside 4 incision
9	187	±19.0	2 elsewhere 2 alongside 1 incision	153.5	±10.0	" "	162.0	±8.0	1 elsewhere 2 alongside 3 incision
10	195	±6.0	4 elsewhere 1 alongside	150.5	±7.0	1 alongside	162.0	±4.0	2 elsewhere 3 alongside 0 incision
11	194	±10.0	All elsewhere	166.0	±13.0	2 alongside 3 incision 4 elsewhere	202.0	±4.4	5 elsewhere 1 alongside 0 incision
12				185.0	±15.0	4 alongside 4 elsewhere			
13				183.9	±7.0	None in incision, 7 elsewhere			
14				206.0	±10.0	All elsewhere			

RESULTS

In the gross, the wounds were well healed and relatively free from peritoneal adhesions. Only one infection occurred in the 65 rats employed. Six animals were discarded because of pulmonary abscesses.



x Mean of observations of adult rat on high protein diet. ———— curve.
• Mean of observations of adult rat on standard diet. ———— curve.
△ Mean of observations of young rat on standard diet. — • — • — curve.
( ) Standard deviation of mean of adult rat on high protein diet.
[ ] Standard deviation of mean of adult rat on standard diet.
o o Standard deviation of mean of young rat on standard diet.

FIG. 1. Curve of tensile strength of wounds in adult and young rats on standard diet and adult rats on high protein diet.

The average strength of the holding power of the sutures in the dead animals was found to be 25 mm. of mercury. The average strength of the wounds in the animals killed 6 hours after operation was 77 mm. of mercury—a difference of 57 mm. of mercury.

The curve plotted from the data (Table II) with the average strength of the wounds in millimeters of mercury as ordinates and days as abscissae (Fig. 1) had the following characteristics: a latent period with the strength remaining practically at a level of 60 mm. for the first 3 days, followed by an increase in strength until the 8th day. During these 8 days all of the ruptures occurred within the suture line, but on the 9th day, both in the suture line and alongside the incision, and on the 10th day, not in the incision but elsewhere in the stomach. This last day was taken as the end-point.

On studying the intake of these rats it was found that they had consumed 11.5 gm. of the diet per 100 gm. of body weight (Table III).

TABLE III

Calculations Based on the Percentage of Body Weight at Start of Experiment

	Young rats (standard diet)		Adult rats (standard diet)		Adult rats (high protein diet)	
	Start to operation	Operation to sacrifice	Start to operation	Operation to sacrifice	Start to operation	Operation to sacrifice
Difference in weight per day, *per cent*	+4.1	+3.1	+0.26	−0.45	−0.3	−0.5
Food consumed per day, *per cent*	12.0	11.0	4.3	2.5	3.3	2.4
Protein consumed per day, *per cent*	2.2	2.0	0.75	0.45	2.6	2.0
Calories of food consumed per day, *per cent*	59.0	55.0	23.0	13.0	14.0	10.0
Calories of protein consumed per day, *per cent*	8.1	7.5	3.1	1.7	9.5	6.8

The daily average consumption of food after operation was approximately equivalent to the average consumption before operation, although on the 4th and 6th days afterwards the average was slightly less than on other days. The rats gained 4.1 per cent of their body weight daily before operation and continued to gain at the rate of 3.3 per cent per day after operation.

Comparison of the Velocity of Healing in the Young and the Adult Rat

The average strength of the holding power of the sutures in the wounds of both the young and adult rats was approximately equal. (25 mm.). The suture techniques were therefore comparable.

In the young rats killed 6 hours after the operation, the wounds

averaged 77 mm. of mercury as compared to 45 mm. in the older group. This increase in the strength after 6 hours, more manifest in the young, could only be attributed to an alteration in the wound occurring almost immediately after suturing. Anatomically, an increased deposition of fibrin was the only demonstrable change. A difference in the amount deposited might account for the greater strength found in the younger animals. The drop in strength from 77 to 50 mm. after the first 24 hours is an expression of the combined loss of strength of the suture material and diminution in the holding

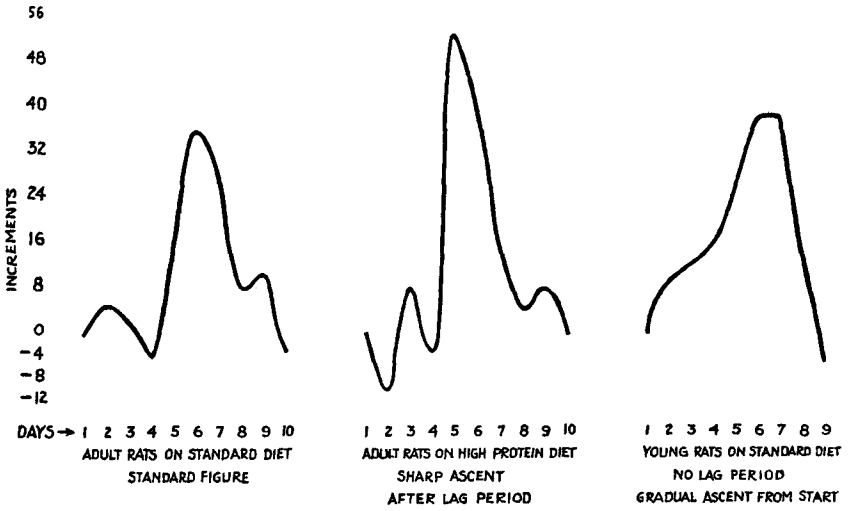


FIG. 2. Curve of daily increments.

power of the tissues. Throughout the latent period an average of approximately 60 mm. was maintained (Fig. 1), and was about 15 mm. higher than that found throughout the same phase in the curve for the adults. Again, as fibrin was demonstrable throughout this latent period and is the scaffold on which the fibrous tissue grows, the greater strength might be attributed to a greater deposition of it. The plus and minus increments of the latent phase, found in all curves, can be explained by the multiplicity of factors influencing the strength of this period.

Once fibroplasia began, however, there were definite increasing positive increments in both curves. This trend began 1 day earlier

in the curve for the young animals, on the 3rd day in contrast to the 4th day in the curve for the adults. The difference was also demonstrable by connective tissue stains. In the wounds of the young rats fibroplastic proliferation was noticeable on the 3rd day while in the wounds of the adults it had not begun on the 3rd day. In spite of this earlier beginning of fibroplasia, its rate in the wounds of the young was no greater than in those of the adults, for the curves were parallel through the 4th, 5th and 6th days. Of course, the 1 day earlier beginning of fibroplasia would account for some shortening of the end-point of healing in the curve for the young animals, but it would not account for the shortening of 3 days which actually occurred, especially when there were equal rates of fibroplasia in the first portion of both curves. The explanation for this is furnished by the length of the accelerated phase. This period lasted from the 3rd through the 7th day in the young rats or 2 days longer than in the adults where it remained from the 4th through the 6th day. Stated in another way, the retarded phase of healing began 1 day later in the curve for the young animals.

Before the end-point was reached, there was in both curves an irregular portion in which both the strength of the stomach and that of the wound was being tested. This period was 1 day shorter in the curve for the younger rats. During this period in the curve for the adults, the strength staggered upwards to reach a strength equivalent to the average strength of the stomachs. In the curve for the young rats by contrast, this equivalent strength was reached before the irregular period began.

Comparison of the Curve for Young Rats with the Curve for Adult Rats on a High Protein Diet

This latter curve was obtained by the same experimental method, except that the rats were fed on a diet containing 68 per cent protein.

The latent period of the curve for the young rats was 1 day shorter than that for the adults on the high protein diet. Once fibroplasia began, however, the daily increments in the curve for the young rats were much smaller than those for the high protein curve, indicating a diminished rate. The entire phase lasted 4 days in the curve for the young as contrasted to 1 in the curve for the adults. In spite of the

larger increment in this phase on the high protein diet, retardation set in 3 days sooner than in the curve for the normal adults. The period of approaching maximum strength began on the 9th day and was of only 1 day's duration in the young in contrast to the 8th day and 3 days' duration in the curve representing the high protein diet. The end-point of the young curve occurred on the 10th day, while for the high protein it fell on the 11th day.

DISCUSSION

The shortening of the period before strength is manifested in wounds of young rats may be compared to similar findings in other fields of biology. Penfold, for example, found a shorter period before the multiplication of bacteria began in the transplants from young parent cultures (6). Robertson (7) noted an equivalent shortening for infusoria taken from young parent cultures. With tissue cultures, Carrel and Ebeling (8), and Burrows (9) obtained quicker proliferation when they employed embryonic serum rather than serum from adults. It seems, then, that young forms in general have a shorter period before reproduction or regeneration begins than adults. This general rule was followed in the wounds of the young animals.

That the rate of fibroplasia is no greater in the wounds of the young than in the wounds of adults seems also to find a counterpart in general biology, where the rates of reproduction of transplants from young cultures, such as bacteria and infusoria, are no greater than in the adult. Robertson (7), commenting on this said, "If, however, the lag-periods [that is, the periods before multiplication begins] be deducted from each, the reproductive rate of individuals isolated from an old parent-culture is not less, but may actually be greater than the reproductive rate of individuals isolated from a young parent-culture."

Without an increased rate of fibroplasia, therefore, shortening of healing time in the wounds of the young must depend on a diminution in the amount of retardation entering into the process. Let us consider how much retardation there is in the curve of healing for the young rats as contrasted to the curves for the adult rats. The onset of retardation in each curve differed (Fig. 2). The phase began 1 day later in the young than in the adults on a standard diet, and 2

days later than in the adults on the high protein diet. The daily amount of retardation calculated by making an algebraic subtraction of each succeeding increment from the one before it shows that the total amount of retardation before the final phase of healing is reached was least in the young (Table IV). For the adult on the standard diet it was 39, for the adult on the high protein 41, and for the young 25. The amount of retardation in the young curve would be even less if it were possible to add the amounts included in the final phase,

TABLE IV

Daily Increments of Strength

Between days	Young curve	Standard curve	High protein curve
1–2	−2 Latent	+6 Latent	−12 Latent
2–3	+12 period	+2 period	+9 period
3–4	+6 Accelerated	−4 Accelerated	−4 Accelerated
4–5	+26 phase	+26 phase	+56 phase
5–6	+38	+36	
6–7	+38 Retarded	+28 Retarded	+41 Retarded
7–8	+13 phase	+8 phase	+15 phase
8–9		+10	
9–10		−3	
Sum of increments in accelerated phase.....	70	62	56
Total amount of retardation. (Calculated by subtracting each succeeding increment from one before and adding differences).....	25	41	39

for the entire final period is shorter in the curve for the young rats. Here is direct mathematical proof, then, that retardation begins later and there is less of it in the healing of wounds in the young.

Next we must consider what is the explanation of the late appearance of retardation in the curve for the young rats. Is it because of a greater rate of fibroplasia during the accelerating phase of healing? Apparently, an increased rate of fibroplasia during the phase of acceleration does not have the capacity to delay the onset of retardation. In the curve for the high protein diet, for example, there were greater

increments during the phase of acceleration, indicating a greater rate of fibroplasia than in any of the other two curves, yet in spite of this, retardation began earlier than in either of the other two. Robertson's theory (7) for retardation in tissue cultures would explain why an early retardation accompanies an increased rate of proliferation. He has presented evidence to show that regeneration and growth are autocatalyzed phenomena. At each cell division, he believes there is a catalyzer given off by the nucleus of the cell, part of which is secreted into the pericellular fluid and part of which remains within the nucleus. As the pericellular fluid becomes saturated with this catalyzer, the amount given off to the surrounding fluid at each cell division becomes less, and retardation occurs as its concentration increases. The end-point is reached when the pericellular fluid becomes saturated. The faster this catalyzer accumulates as a result of rapid proliferation, the sooner retardation occurs. This theory would explain why there is early retardation in the high protein curve and why there is late retardation in the curve for the young. It would also strengthen the viewpoint that there is a less rapid rate of proliferation in the healing of wounds of the young.

Carrel's and Ebeling's experiments (8) showed that age played a rôle in retarding the proliferation of cells. Their tissue cultures in embryonic serum came to a greater mass in a shorter length of time than in the serum of adults. When they diluted the embryonic serum to twice its volume, the rate of proliferation was not decreased. A diminution should have followed if the embryonic serum contained an accelerating substance. They therefore concluded that there must be an inhibiting substance in the serum of the adults. The presence of an inhibiting substance in the blood of the adult rat would explain why there was an early appearance of the retarded phase in the healing of their wounds.

The combination of retardation arising from the age factor and from autocatalysis would adequately explain all the variations in retardation found in the three curves. In the adults on the normal diet, retardation from age added to autocatalytic retardation would act to slow the process earlier than in the young where there was no retardation from age. In the adults under the stimulation of the high protein diet, the greater autocatalytic retardation plus the retardation from age would slow the process even earlier than in the adults on the

standard diet and the total amount of retardation would be greater. In the young, all retardation attributable to age could largely be deducted and there would remain only autocatalytic retardation. Accordingly, it should appear later and the total amount should be less. This is actually what is found in these experiments.

Of course, the completion of the healing of the wound is a balance of both the duration and quantity of the accelerated and the retarded phases, and the relation of these phases in the curve for the adult rats on the standard diet must be taken as the norm for the comparison of the phases in the other two curves. A review of the balance of the two phases in comparison to the standard curve will show why the healing times varied. Complete healing was obtained more rapidly on the high protein diet in spite of more retardation because the accelerated phase was quantitatively greater during its existence. The end-point of healing was reached more rapidly in the curve for the young rats not because of greater acceleration either in duration or quantity but because retardation appeared later and was less in quantity.

Naturally, it can be argued that in the curve for the young rats there was an increased rate of fibroplasia—that the increments during the first part of the curve are only equal to those of the adult because retardation has already reduced them to a seeming similarity. However, this argument does not hold for if it were applicable to the young curve it would be equally so for all the curves. We must reason, therefore, from the time at which the increments definitely begin to change.

In all three experiments an effort was made to produce a wound of approximately the same size and to suture it in the same way. Theoretically then, the same amount of fibrous tissue was regenerated and the algebraic sum of the combined forces of acceleration and retardation working antagonistically to complete healing should be approximately the same in all three experiments. How near this was approached can be seen in the following tabulation:

Total accelerating increments.....	Young 70	Standard 62	High protein 56
Total retarding increments.....	" 25	" 39	" " 41
Addition.....	95	101	97

The discussion of retardation in the curves of the healing of the wound inspires the question as to what there is that limits scar tissue in the process of regeneration. There is little evidence as to what this may be, but there is much evidence as to what it is not. The work of Carrell (8) suggests that it is referable to the blood plasma. If so, it is not the exhaustion of food supply, for surely it remained the same in all three experiments. It is apparently not an accumulation of by-products, for this idea has been refuted many times as an explanation of why bacteria in media when they reach a definite number stop growing. Hixson (10) found that his yeast cultures repeated their growth cycles if the alcohol were drawn off. It if is local or more intimately related to the cell itself, it is not the exhaustion of the capacity of the cell to reproduce itself. Carrel's (11) tissue cultures of heart muscle under favorable conditions for growth have proliferated for years. Moreover, Osborne and Mendel (12) were able to delay growth by restricting food until the animal was quite old, but then growth was renewed with equal vigor when food was again given. Here the capacity for regeneration was not lost, even though the supply of food was insufficient. We know, too, that the cicatrix has the capacity to regenerate again and again, for it will do so in response to many new injuries. Therefore, the ability of the cells to regenerate is not lost, in the arrest of fibroplasia. If the retardation is physical, *i.e.* compression on the cells after a certain mass is produced, then it must be remembered that spacial relations are no barrier to keloids, to simple hypertrophies or to malignancies. These last two conditions are, of course, abnormal manifestations of proliferation of tissues, but they must be considered here for tumefaction must be caused either by an absence of the retardation or an increased rate of proliferation.

The caloric intake of the young rats was practically four times that of the adult (Table III). Consequently, the amount of protein taken by them as calculated in relation to the body weight was high, in fact equal to that of the adults on the high protein diet. Yet, this relatively high protein and caloric intake did not increase the rate of fibroplasia as it did in the adults on the high protein diet. That this high protein, high caloric intake was utilized by the young for growth, daily maintenance and wound healing, while in the adults it was used simply for daily maintenance and wound healing, seems to furnish a

logical explanation of why there was not an increased rate of fibroplasia in the young, especially as even the young controls ate four times as much as did the adult controls. The young rats continued to gain in weight after operation at a rate only slightly less than before. Under similar conditions, the adult rats lost a slight amount.

There is then good evidence to suggest that the shortening of the healing time in young animals is not because of an increased rate of fibroplasia but rather from a diminution of factors retarding the process. Of course, in the final analysis of the entire process of the healing of wounds of the same size in both the young and old, the total amount of retardation must be the same in order to stop both processes. However, in the young animals for the phase of healing tested by this method, a diminished amount of retardation and its late appearance combined with an earlier appearance of fibroplasia allow earlier reacquisition of strength and nothing can be said about the amount of retardation entering into the final organization of the cicatrix.

SUMMARY

The velocity curve of fibroplasia in the healing of wounds in young rats reached its end-point 3 days ahead of a similar curve for adults. Strength and fibroplasia were manifest 1 day sooner than in the adults. A study of the increments of the curve showed that the rate of fibroplasia during the accelerated phase was less in the young and that it lasted longer. Correspondingly, retardation appeared later and was less in amount than in the curve for the adult rats. The amount of retardation was even less than in the curve obtained for adults on a high protein diet, in spite of the fact that in this latter curve there was a definite increase in the rate of fibroplasia.

Healing in the young, therefore, is more rapid than in adults because fibroplasia begins earlier and is less retarded, not because the rate of fibroplasia is greater.

Growth of the young is not hindered by the process of wound healing.

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