Uptake of Radiosulfur During the Healing of Surgical Wounds

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The fact that sulfur metabolism is altered markedly during the regeneration of wound tissue (1-7) has led us to investigate aspects of this phenomenon which might serve as the basis of a possible technic for measuring the rate of healing in humans. It has been shown that the sulfur amino acids, methionine and cystine, are accumulated in regenerating wound tissue (6). The rate of deposition, as well as the relative amount of cystine in the wound tissue, is much different from that of methionine; the level of cystine in the wound tissue is appreciably higher than that in other tissues (4, 6). Further, there seems to be evidence that indicates a correlation between the concentration of cystine in wound tissue and the relative amount of healing—as measured by rupture strength of the wound (2, 5).

The rate of healing is affected by many circumstances. Not all of these are known and, hence, cannot be carefully controlled in experimental situations. This inability to control extraneous factors which affect the rate of healing usually leads to large variations in the assessment of the parameter which is measured as the function of time. To some extent this difficulty may be overcome by the use of sufficiently large numbers of experimental subjects. However, this type of solution of the problem is in itself a severe limitation on the use of humans in experiments on the healing of wounds. The problem of variability of the experimental subjects may be largely compensated for by having each serve as its own control. However, prac-
tically all of the valid technics described for determining the rate of healing involve measures which are destructive of the regenerating wound tissue (8–11). This type of determination precludes the use of an animal as its own control, since the healing processes must begin afresh after each intervention into the healing wound tissue. This may also be considered a further limitation on the use of humans in wound-healing experiments.

A technic which involves nondestructive methods of measurement to a large extent could obviate the pertinence of both the above difficulties. Since the sulfur amino acids are deposited in regenerating wound tissue, it was considered that the measurement of radiosulfur-labeled amino acids in the wounds might be used as the basis of a nondestructive technic. This sort of procedure, of course, would be based on the measurement of the radiosulfur at the surface of the wound. In this way consecutive measurements of radioactivity at the surface of the same sample of wound tissue could be made after varying intervals of time, without disturbing the healing processes taking place in the sample. The validity of such a technic rests on the assumption that the activity of S\textsuperscript{35} at the surface of the wound is a constant function of the concentration of S\textsuperscript{35} throughout the regenerating wound tissue. This report presents the results of studies on the deposition of systemically administered S\textsuperscript{35} in the surface of wounds in humans.

**EXPERIMENTAL**

The data in this paper were obtained primarily from measurements on humans who had undergone subtotal gastrectomy for benign duodenal ulcer. A lumbar sympathectomy to relieve peripheral arterial occlusion was performed on a very few subjects included in these data. Both operative procedures gave essentially the same results in these experiments. These surgical procedures were chosen since (1) the injury inflicted would be expected to be of sufficient severity to elicit a maximal metabolic response in the experimental subjects, and (2) the patients would remain available for a time sufficient to permit the experiment to be carried out under the relatively controlled conditions of the hospital. In all, 25 subjects were used in the following experiments.

Each experimental subject was given 1 mc. of radioactive sulfur in the form of either cystine, methionine, or sulfate; the S\textsuperscript{35} was given either 24 hours preoperatively or about 24 hours after the operation.
Although attempts were made to measure the $S^{35}$ activity within less than 24 hours after operation, this was not usually successful because of the exudation of serum or because of minor bleeding from the incision. Therefore, measurements of the $S^{35}$ activity were begun routinely about 24 hours postoperatively and were carried out daily thereafter for about one week. After this time, measurements were made at longer time intervals. In general, the area of the wound chosen for measurement presented the picture of a "fine-line incision" with well-coapted edges and with minimal inflammation. Gaping or inflamed sections of the wounds gave random fluctuations of the $S^{35}$ activity. After thorough washing to remove dried serum, blood, etc., two areas of the incision and a distant skin area were measured for $S^{35}$ activity. The left lateral upper thigh usually was the site used for determining the activity in the skin.

Since $S^{35}$ is a low-energy beta emitter (0.167 Mev), it was considered at first that a highly sensitive measuring instrument would be required. For this purpose, a thin, end-window gas-flow counter was developed. It was soon found that the movement and handling of this detection device required for setting up each experiment introduced various artifacts which made the measurements not entirely reliable. The substitution of a Geiger-Müller tube (window = 1.4 mg./sq. cm.) for the gas-flow counter appeared to decrease the sensitivity by only a relatively small extent in our experimental system and to give very much more reproducible results.

To obtain reproducible geometry during successive measurements of the same site on the wound or skin area, a 1.3-mm.-thick brass reference plate (RP) was devised, as illustrated in Fig. 1. The RP was placed in position in such fashion that the incision was centered in the slot, $D$. The RP then was firmly attached to the skin by applying adhesive tape with a small amount of pressure across the lateral wings, $F$. The degree of pressure applied to the RP was inconsequential since the same amount of tissue would always "pout" through the slot. This made it possible to achieve a constant wound-to-window distance for every measurement. The position of the RP over the wound or the skin was marked with an indelible pencil so that the same positions could be found for the subsequent determinations of the $S^{35}$ activity in the same part of the wound. The Geiger-Müller tube, $A$, was permanently positioned in a protective Lucite tube, $B$, by means of set screws. To measure the $S^{35}$ activity, the Lucite tube with the G-M counter in it was mounted on the pin and
against the positioning block, C, of the RP. The lucite tube was held securely in place over the slot, D, by means of a coiled steel spring, E. The cable connecting the G-M tube assembly to the scaler was held in position by flexible steel tubing so that there was freedom of movement in three directions. This was of particular importance to compensate for the respiratory movements of the subjects without displacement of the RP or the G-M tube assembly during the time when the $^{38}S$ was being measured.

Determinations were made of the $^{35}S$ activity with the RP positioned at various distances from the surgical wound. The activity dropped off very sharply when the slot of the RP was moved even a small distance from the incision. As can be seen in Fig. 2, when the RP was moved so that none of the wound was exposed, but the...
incision was only 3 mm. from the edge of the slot, the S\textsuperscript{35} activity decreased essentially to the level observed in the skin of the thigh. This occurred only when a fine-line incision with minimal inflammation was being measured. The areas directly adjoining inflamed wounds usually had more than 50 per cent of the activity of the regenerating wound tissue.

The slot in the RP being appreciably wider than the width of the regenerating wound tissue in the incision, a correction had to be made to account for the activity in the nonregenerating tissue being measured at the same time as that in the wound tissue. The correction was made by the use of the following equation:

\[ C_w = k \left( C_t - \frac{A_t - A_w}{A_t} C_s \right) \]

where \( C_w \) is the counts per minute per 100 sq. mm. in the regenerating wound tissue, \( C_t \) the counts per minute measured over the wound area, \( C_s \) the counts per minute measured over the nonwound area, \( k \) the factor to adjust values to counts per minute per 100 sq. mm., \( A_t \) the total area of the G-M window unshielded by the slot in the RP, and \( A_w \) the area of the wound being measured. All of the values of S\textsuperscript{35} were corrected for background and decay before being used in the equation. Each measurement (\( C_t \) and \( C_s \)) was made for 1280 counts or at least 10 minutes.

RESULTS AND DISCUSSION

Figure 3 shows the change in S\textsuperscript{35} activity with time in the surface of wounds after the preoperative administration of methionine-S\textsuperscript{35}. The activity reaches a peak level about 4-6 days after the wound was

![Graph showing change in S\textsuperscript{35} activity over time.](image)

*Fig. 3. $S^{35}$ activity measured in surgical wounds and skin of subjects to whom methionine-$S^{35}$ was administered prior to operation. Labeled compound was given on day 1 and surgery carried out on day 2.*
made and then gradually decreases over a period of 2-2½ months to the level of activity found in the skin. It should be noted that about 5 times as much radiosulfur is found in the surface of the wound tissue as can be measured in the surface of the skin within a short time after the operation. In the subjects who received the methionine-S₃⁵ after surgery (Fig. 4), the highest level of S₃⁵ activity in the wound was not reached until about 10-16 days after the administration of the labeled compound; the activity in these wounds decreased to the level in the skin in about 2-2½ months also.

The maximal concentration of S₃⁵ activity in the wounds of the subjects given the methionine-S₃⁵ after operation is significantly higher than in the wounds of those receiving the S₃⁵-labeled compound prior to surgery. This is due, at least in part, to the metabolic concomitants of the processes involved in the healing of the wounds. Some part of the preoperatively administered S₃⁵ is excreted, either directly or after metabolic interchange and alteration in the body tissues, even before the wound is inflicted. On the other hand, the S₃⁵ administered after surgery is available for deposition in the wound tissue at once. Further, it has been shown that there is a decreased excretion of sulfur-containing compounds after wounding (1, 2), so that one might expect a greater conservation of the S₃⁵ given postoperatively. The end result of all these circumstances is that the effective amount of S₃⁵ available for deposition in the wound tissue is less when the labeled methionine is given before wounding than after, even though the actual amount of labeled compound administered is the same in both situations. The amount of S₃⁵ in-

Fig. 4. S₃⁵ activity at surface of surgical wounds and skin of subjects who received methionine-S₃⁵ after operation. Surgery was carried out on day 0 and labeled compound given on day 1.
corporated into the wound tissue is expected to be a function of the size of the effective dose of the labeled compound. Probably also related to the difference in the amount of S\(^{35}\) available for the wound is the observation that the maximal level of S\(^{35}\) is reached sooner in those subjects receiving the methionine before operation than in those receiving it after.

The results of similar experiments, using cystine instead of methionine as the source of S\(^{35}\), are shown in Fig. 5 and 6. The pattern of results obtained was the same with the cystine-S\(^{35}\) as with methionine-S\(^{35}\). In these experiments, also, the maximal level of S\(^{35}\) activity in the wounds of the subjects given the cystine-S\(^{35}\) prior to the operation was significantly less than in those given the labeled cystine after operation. Again, when cystine-S\(^{35}\) was administered preoperatively, the peak S\(^{35}\) activity in the wound appeared within 2-3 days after operation, as compared with 6-8 days when given postoperatively. Undoubtedly, the explanation for these differences is
the same as that for the differences observed when methionine-S\textsuperscript{35} was the source of the radiosulfur.

A comparison of the data from the experiments in which both labeled cystine and methionine were administered preoperatively (Fig. 3 and 5) shows that the S\textsuperscript{35} activity in the wounds of the subjects receiving the former is significantly less than in those receiving the latter. The same situation holds with a comparison of the results of postoperatively administered cystine-S\textsuperscript{35} and methionine-S\textsuperscript{35} (Fig. 4 and 6). These differences depend on the fact that the conversion of methionine to cystine is irreversible in vivo (12, 13). Thus, when methionine-S\textsuperscript{35} is administered there are two sources of S\textsuperscript{35} available, methionine-S\textsuperscript{35} and its metabolic product cystine-S\textsuperscript{35}, each supplying a specific set of independent requirements in the regenerating wound tissue; the cystine-S\textsuperscript{35} can serve only as a single source.

When sulfate-S\textsuperscript{35} was given, only an exceptionally small amount of activity was measurable (Fig. 7). This activity very soon disappears (about 14-20 days) from the surface of the wound. In contradistinction to when methionine-S\textsuperscript{35} or cystine-S\textsuperscript{35} is administered (following which the low level of skin-S\textsuperscript{35} activity persists long after all the S\textsuperscript{35} has disappeared from the wound), after sulfate-S\textsuperscript{35} is given the activity in the skin disappears within 12 days. Administration of the sulfate-S\textsuperscript{35} before or after wounding resulted in no measurable difference in the amount of S\textsuperscript{35} in the regenerating wound. It seems probable that a large part of sulfate-S\textsuperscript{35} is excreted, because of its great diffusibility, before much of it can be utilized in the tissues for metabolic purposes. It has been shown that sulfate-S\textsuperscript{35} is readily taken up by regenerating wound tissue for the formation of sulfated mucopolysaccharides (14-17). Since these polysaccharides are part of the ground substance and intercellular fluid matrix of the wound

![Graph](image_url)

**Fig. 7.** S\textsuperscript{35} activity at surface of surgical wounds and skin of patients given sulfate-S\textsuperscript{35}. 
tissue (18), the S\textsuperscript{35} deposited in these compounds would be expected to be measurable by our technic only during the earliest stages of healing and before the collagen and cellular elements in the wound have become compacted into a relatively dehydrated layer which contains little, if any, ground substance and which acts as a barrier to the weak beta radiations emitted by the S\textsuperscript{35}.

**SUMMARY**

The feasibility of using the deposition of S\textsuperscript{35} in wounds as a measure of the rate of healing was investigated by administering isotopically labeled methionine, cystine, or sulfate to surgical patients. It was found that the level of S\textsuperscript{35} measurable at the surface of the wounds was higher when either methionine-S\textsuperscript{35} or cystine-S\textsuperscript{35} was given after wounding than when given before. More S\textsuperscript{35} was incorporated into the wound tissue when methionine was the source of the isotope than when labeled cystine supplied the S\textsuperscript{35}. About 4-7 times as much S\textsuperscript{35} was detectable in the regenerating wound tissue within a few days after wounding, compared to S\textsuperscript{35} detectable in unaltered skin. Only a relatively small amount of sulfate-S\textsuperscript{35} was taken up by healing wound tissue. These results were obtained by the use of a technic which made it possible to measure repetitively, over a long period, the activity at the surface of the same sample of wound tissue.

**REFERENCES**