# IRON EXCRETION BY THE SKIN. SELECTIVE LOCALIZATION OF IRON$^{59}$ IN EPITHELIAL CELLS

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There have been numerous publications reporting the presence of iron in human sweat. The concentration and amount of this element has varied greatly, however, with the method of stimulation and collection of the sample.[1-7] Thus the quantitative significance of iron loss from the skin is still unknown. The present study was undertaken in an attempt to demonstrate the method by which the skin acts as an active excretory organ for iron and its importance in maintaining iron balance.

## MATERIAL AND METHODS

Ferrous$^{59}$ citrate, 0.5 $\mu$c per 0.15 $\gamma$, was injected intravenously in a healthy iron replete volunteer. Thereafter multiple determinations of the retention of iron$^{59}$ were made over a 100-day period by placing the subject into a human whole-body liquid scintillation detector. Cumulative stool and urine collections were made during this period. The specimens were brought to a constant volume and the radioactivity counted in the whole-body liquid scintillation detector. This was compared to a standard consisting of the exact intravenous dose in a similar volume.

Ferrous$^{59}$ citrate, 1.5 $\mu$c per 0.1 $\gamma$ per 0.05 ml, was injected intradermally into the forearms of 6 iron-replete volunteers. Subsequent determinations of radioactivity were made by placing the subject's arm in a small animal whole-body liquid scintillation detector (Packard ARMAC). The non-injected arm was also counted and served as the background, thus eliminating counts from iron in the circulating blood.Aliquots (2 ml) of plasma and whole blood were counted in a well-type NaI crystal scintillation detector (Packard).

Biopsy specimens, obtained from the forearm on the 14th day following the intradermal injection of the isotope, were fixed in formalin and embedded in paraffin. Sections were mounted on slides and then covered with photographic emulsion (Kodak NTB-3). The slides were developed after a 40-day exposure and then stained with hematoxylin and eosin. Prussian blue reaction was used to stain for iron and the tissue counterstained with picric acid.

## RESULTS

### *Total Body Loss*

Following an intravenous tracer dose of iron$^{59}$ there was an initial rapid loss in the stools in the first 2 to 3 days (Text-fig. 1). Thereafter

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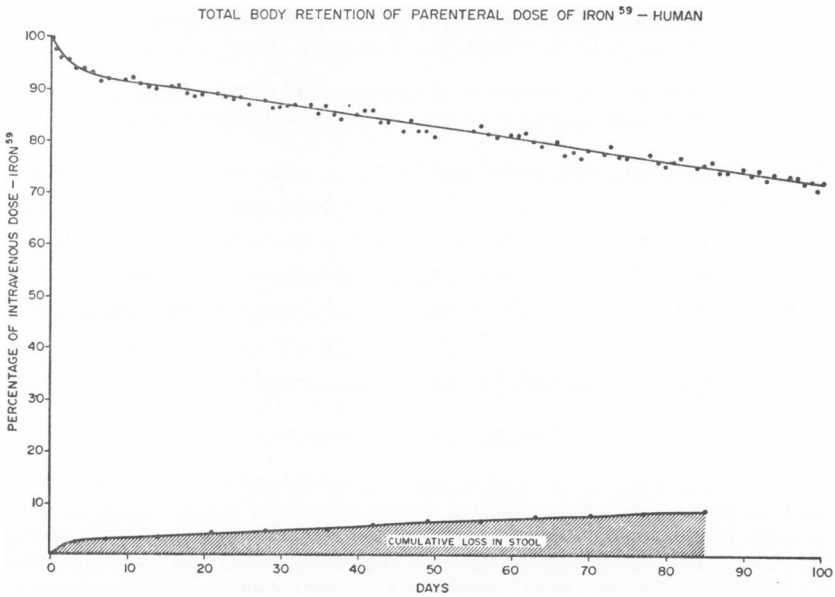
there was a rate of loss which remained constant throughout the 100-day period. This amounted to 0.22 per cent per day of the initial dose. Excluding the first 3 days the rate of accumulation of iron[59] in the stools was 0.08 per cent per day of the initial dose. During 85 days (including the first 3 days) a total of 8.5 per cent of the initial dose was re-




TEXT-FIG. 1. Total body retention and cumulative stool recovery of a parenteral dose of iron[59] in a normal subject as measured in a human whole body liquid scintillation detector.

covered in the stool. However, loss as measured by whole-body counts was 25 per cent. A cumulative collection of the first week's urine had no significant radioactivity.

### *Skin Studies*

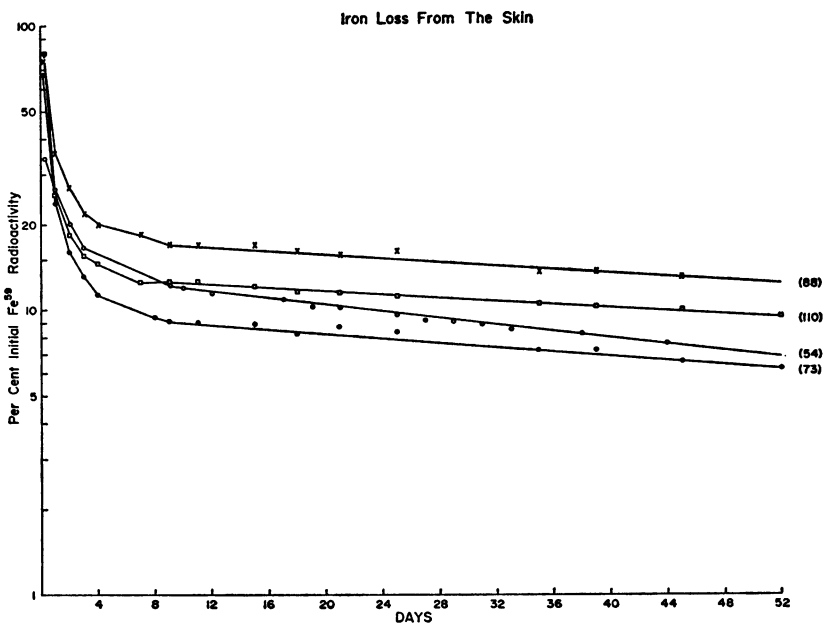
In the first 2 days after the intradermal injection of iron[59] there was a 75 to 85 per cent fall in radioactivity of the arm with a concomitant rise and fall of the plasma radioactivity (Text-figs. 2 and 3). Subsequently there was a slow second rate of loss. Corrected for decay, the time required for half the radioactivity to leave the skin varied from 54 to 110 days in four of the subjects. External loss of iron was demonstrated during this period of time by the presence of significant radioactivity in heat stimulated sweat collections and Scotch tape keratin strippings from the forearm. In 2 additional volunteers the site of injection was excised on the 14th day which resulted in a 20 per cent and 42 per cent drop in the radioactivity in the arm. Radioautographs prepared from sections of the skin showed selective localization in the epithelial cells of the epidermis, eccrine sweat gland, sebaceous gland, and hair

follicle (Figs. 1 to 5). There was no significant radioactivity in the connective tissue, despite the fact that this was most likely the site of the tip of the needle at the time of injection.

The radioactivity in the epidermis was most prominent in the basal layer of the stratum malpighii and decreased toward the stratum granulosum. The concentration of cells per unit area is greatest in the basal layer and accentuates the gradation in radioactivity noted. In the hair follicle the iron[59] was primarily in the outer root sheath. The secretory (coiled) tubules of the eccrine gland appeared to have more radioactivity than the ductal epithelium. In the sebaceous gland the activity was greatest in the periphery of the gland.

## Discussion

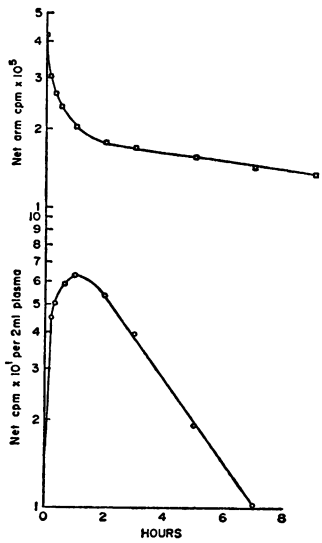
The loss of whole body radioactivity following an intravenous dose of iron[59] is approximately 3 times greater than the accumulation of




Text-fig. 2. Retention of iron[59] in the skin following an intradermal injection on the forearm. The number in parentheses is the half life in days of the second phase of the curve for each subject.

radioactivity in collections of feces and urine. The remaining possible pathway for excretion is through the skin. After the intradermal injection of trace amounts of iron[59] there was a rapid disappearance of radioactivity from the arm during the first day. This was due to uptake by the local blood supply as demonstrated by the concomitant increase of radioactivity in the plasma. In the subsequent weeks there was a

second, slow loss of iron$^{59}$. During this period we were able to demonstrate selective localization of the iron$^{59}$ in the epithelial cells of the epidermis and glands of the skin. The presence of radioactivity in old as well as new cells indicated that iron could be accepted by such cells




TEXT-FIG. 3. Simultaneous determinations of radioactivity in the arm and plasma of a subject following the intradermal injection of iron$^{59}$.

after they were formed. Loss of iron can occur with desquamation of these cells and with secretion of sweat. The former is most likely the major pathway since the iron content of cell-rich sweat is significantly greater than cell-free sweat.[4–7] Iron$^{59}$ detected in collections of sweat and keratin suggested that external loss of iron was responsible for a part of the second phase of the decrease in radioactivity of the arm.

The next thing to be considered is whether the incorporation of iron into the epithelial cells and subsequent loss was a passive or active excretory process. It is possible that the radioactivity we were seeing was due only to iron incorporated into cells for vital enzyme function. If this was true one would not expect to see increased iron deposition in these cells in iron-loaded patients. Sections of a skin biopsy from the forearm of a patient with hemochromatosis were stained to give the Prussian blue reaction. The selective distribution of iron in the epithelial cells of the epidermis and the sweat glands was similar to that seen in the radioautographs from the normal volunteers (Figs. 6 and 7). Intracellular iron which reacts to form Prussian blue is in excess of any physiologic requirement of the cell. These findings support the concept that the iron accumulation in epithelial cells may represent an active excretory process.

Our studies suggest that the skin plays a major role in the daily loss of iron. The radioactive iron data cannot be exactly quantitated since we do not know the size of the pool of epithelial iron from which loss takes place. The surface area of an average adult is 1.73 sq m. This figure is significantly increased by adding the exfoliative surface of the epidermal appendages (sweat glands and hair follicles) which number in excess of 130 per sq cm.[8] In comparison the exfoliative surface of the small intestine (villous tips) is approximately 1.2 square meters. One must also consider the turnover rate of the cells in the gut and the skin. The epithelium of the intestinal villus is replaced every 3 days[9] and this rate may be faster than that of the skin (epidermis, 26 days[10]; hair matrix, 1 day[11]; root sheath and sweat glands, unknown). However, the larger surface area of the latter plus the contribution of iron loss in the secretory process might account for the greater loss of iron[59] from the skin than the gut. Thus the skin as well as the small intestine[12] can function as an excretory organ for iron through the loss of iron-loaded epithelial cells. The property of these cells which enables them to sequester iron remains to be determined.

## Summary

Following an intravenous dose of iron[59] loss of whole body radioactivity was significantly greater than could be accounted for in cumulative collections of stool and urine. Selective localization of iron in the epithelial cells of the epidermis and its appendages with subsequent external loss was demonstrated in normal volunteers with the aid of radioautography. That this was an active excretory process was supported by the finding of stainable iron with a similar distribution in the skin of a patient with hemochromatosis. Thus the skin as well as the small intestine functions as an excretory organ for iron through the loss of iron-loaded epithelial cells.

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## LEGENDS FOR FIGURES

Except where indicated photomicrographs were prepared from sections stained with hematoxylin and eosin.

FIG. 1. Radioautograph of normal skin biopsy following the intradermal injection of $iron^{59}$. There is selective distribution of radioactivity in the epithelium of the epidermis and skin appendages. No significant accumulation of $iron^{59}$ appears in the connective tissue. × 30.

FIG. 2. $Iron^{59}$ radioautograph, normal epidermis. The radioactivity in the epidermis is most prominent in the basal layer of the stratum malpighii and decreases toward the stratum granulosum. × 105.

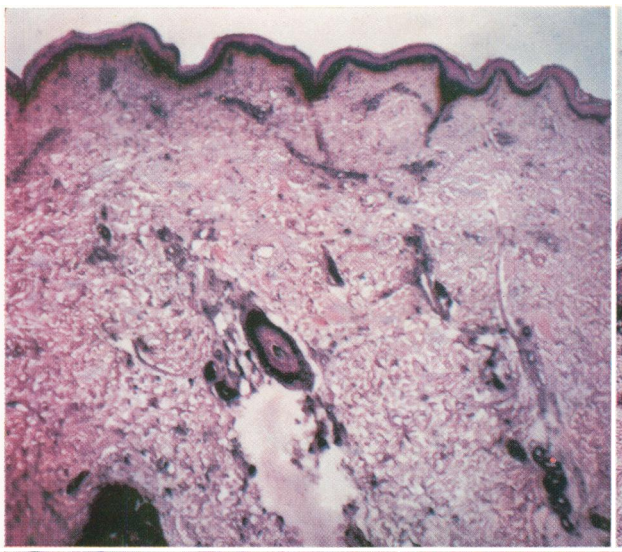
FIG. 3. $Iron^{59}$ radioautograph. Selective localization of radioactivity is seen in the coiled tubules of the normal eccrine sweat gland. × 105.

FIG. 4. $Iron^{59}$ radioautograph. $Iron^{59}$ is distributed in the outer sheath of the normal hair root. × 105.

FIG. 5. $Iron^{59}$ radioautograph. The concentration of $iron^{59}$ is greatest in the epithelial cells at the periphery of the normal sebaceous gland. × 105.

FIG. 6. Skin biopsy, patient with hemochromatosis. The distribution of iron, as demonstrated by the Prussian blue reaction, in the epithelium of the epidermis is similar to that of the $iron^{59}$ in the radioautographs of skin from normal volunteers. Potassium ferrocyanide stain. × 105.

FIG. 7. Cross section of an eccrine sweat gland tubule, the skin biopsy from a patient with hemochromatosis. Iron, demonstrated by the Prussian blue reaction, is seen within the epithelium. Potassium ferrocyanide stain. × 624.



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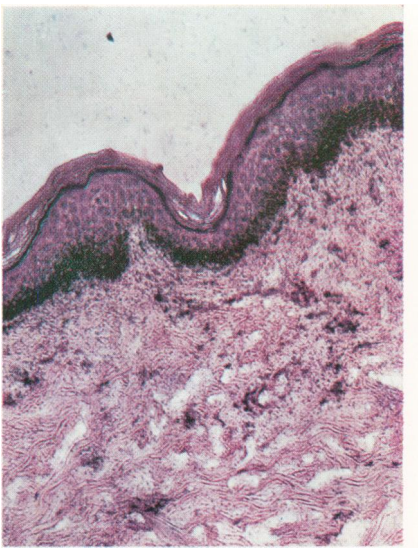

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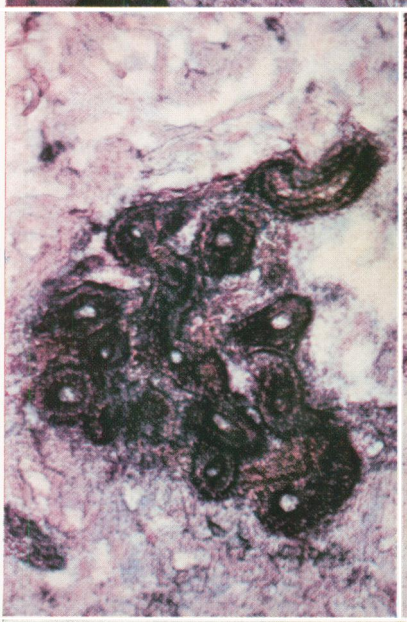

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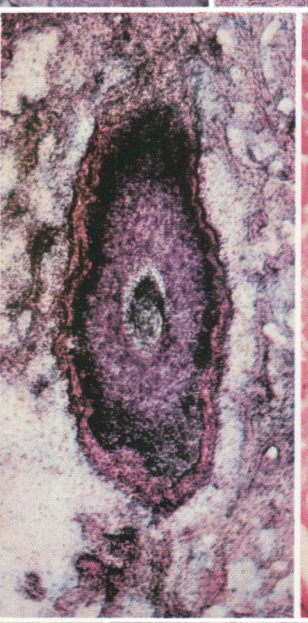

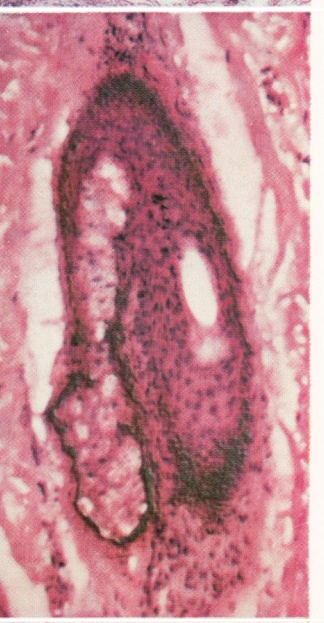

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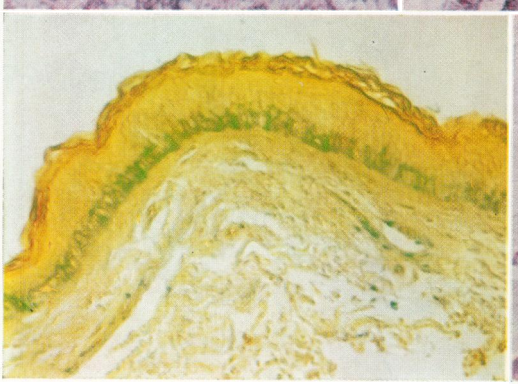

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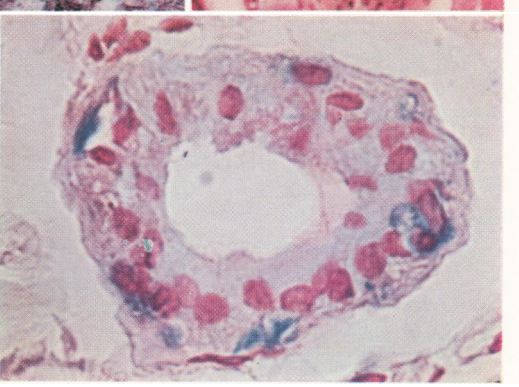

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