

TURNOVER OF S³⁵-SULFATE IN THE MUCOSA OF THE
GASTROINTESTINAL TRACT OF RATS AS SEEN
IN AUTORADIOGRAMS

By DOMINIC D. DZIEWIATKOWSKI, Ph.D.

(From *The Rockefeller Institute for Medical Research*)

PLATES 7 TO 9

(Received for publication, November 17, 1955)

Using autoradiography Odeblad and Boström (1) found that sulfur-35 had been localized to a greater extent in the mucosa than in the underlying coats of the intestinal tract of rats given labelled sulfate. They suggested that sulfate is utilized in the mucosa for the synthesis of sulfated polysaccharides such as mucoitin sulfuric acid. Bélanger (2, 3) described the ingress and exit of S³⁵-sulfate in different regions of rats' and hamsters' stomachs, and confirmed the observations of Davies and Young (4) that mucus-secreting cells of the salivary glands exhibit a transient but intense over-all uptake of the isotope. A recent report by Boström and Odeblad (5) emphasized the rapidity with which the intestinal mucosa handles labelled sulfate, but according to Bélanger (3) not all the mucosal cells are involved, the goblet cells but not the Brunner glands being active.

The above suggestions in regard to the rapid turnover of sulfate in the gastrointestinal tract are confirmed in a study (6) in which sulfated polysaccharides were isolated from the viscera of rats at intervals of time following the intraperitoneal injection of S³⁵-sulfate: the specific activity of the sulfate of the isolated materials increased and then decreased rapidly. Subsequently an attempt was made to determine by autoradiography the regions of the intestinal tract in which this rapid turnover of sulfate occurred. The results confirm those reported by Bélanger (3) and Boström and Odeblad (5). It is concluded that in the intestine, the mucosa rapidly accumulates more of the sulfur-35 supplied to the animal and then also frees itself of most of it more rapidly than the rest of the intestinal tissue. Coated autoradiograms reveal, in addition, a fact previously unobserved, namely, that the goblet cells that are farthest from the intestinal lumen accumulate and lose the sulfur-35 sooner than those cells closer to the lumen.

Materials and Methods

Male rats, 9 to 13 months old and weighing 275 to 325 gm., were each given an intraperitoneal injection of 250 microcuries¹ of carrier-free sulfur-35 as sodium sulfate in 1 ml. of water.

¹The sulfur-35 was supplied by the Oak Ridge National Laboratory on allocation from the United States Atomic Energy Commission.

Three rats were then sacrificed 6, 24, and 48 hours later by exsanguination under ether anesthesia. Segments were removed from various levels of the gastrointestinal tract and fixed in 10 per cent formalin for 48 hours. Following a wash in water for 24 hours, the tissues were dehydrated in alcohol of increasing concentrations, remaining in each different concentration for 24 hours. After imbedding in paraffin, sections 7 μ thick were cut. Contact autoradiograms were prepared as previously described (7) and coated autoradiograms were prepared according to Gross *et al.* (8), using Eastman Kodak NTB₂ emulsion.

RESULTS AND DISCUSSION

In all the autoradiographic studies cited in the Introduction the tissues were fixed in organic solvents, wherein inorganic sulfates would be expected to have only a limited solubility. On the basis of the autoradiograms produced by sections of tissue thus fixed it is not possible to decide to what extent the autoradiographic reaction is due to inorganic sulfate on the one hand or to bound sulfate on the other. This is particularly true in the case of the specimens removed shortly after administration of the S³⁵-sulfate, that is, at a time when most of the injected inorganic sulfate is circulating as such in the organism. To minimize this difficulty in the present study the tissues were fixed in aqueous formalin and were then washed in water. Even under these conditions, as is seen in Figs. 1 to 12, a rapid accumulation of sulfur-35 is demonstrable in the walls of the gastrointestinal tract up to about the 24th hour, followed by a rapid disappearance of the major fraction of the isotope by the 48th hour. The uptake of the S³⁵-sulfate varies at different levels of the intestinal tract and can indeed be correlated with the numbers of mucus-secreting goblet cells at the different levels.

The coated autoradiograms reproduced in Figs. 13 to 17 and Figs. 18 to 23 show in greater detail the autoradiographic reactions given by segments of the intestinal tract at an upper and lower level. In the duodenum 6 hours after the administration of the labelled sulfate it is seen (Fig. 13) that the punctiform reaction is most pronounced at the level of the crypts of the mucosa. The mucus in the lumen shows very little activity. By the end of 24 hours (Fig. 14) the radioactivity is the greatest at the level of the villi and the mucus in the lumen is highly active. At the end of 48 hours (Fig. 15) only a diffuse and faint reaction is given by the intestinal mucosa and the mucus in the lumen has very little of the sulfur-35.

The concentration of sulfur-35 by the mucosa of the colon is many fold greater than that in the duodenum. Examination of these autoradiograms revealed that the punctiform arrangement of the silver grains corresponded to the arrangement of the underlying goblet cells. This is illustrated by Figs. 21 to 23, which are photomicrographs of stained sections adjacent to unstained sections used to produce the autoradiograms reproduced as Figs. 18 to 20. It is then apparent in Figs. 16 and 18 that by the end of the 6th hour interval the goblet cells that are deepest in the glands of Lieberkühn have accumulated the greatest amount of the isotope. At the end of 24 hours, a more uniform and a more

intense autoradiographic reaction is given by the goblet cells (Figs. 17 and 19) and the mucus in the intestinal lumen is highly radioactive. By the end of 48 hours the lower intestine also gives only a diffuse and faint autoradiographic reaction (Fig. 20).

SUMMARY

Segments of the gastrointestinal tract removed from rats after intervals of time following injection of S^{35} -sulfate were fixed in aqueous formalin and then washed in water. Contact and coated autoradiograms were prepared. The suggestion made by others that more of the labelled sulfate is fixed by the mucosa than by the underlying coats of the gastrointestinal tract is confirmed. In addition it was found that the isotope is fixed to a greater extent in the lower intestine than in the middle or upper portions of it.

Coated autoradiograms revealed that 6 hours after administration of S^{35} -sulfate more of the label was present in the goblet cells lying deep in the crypts of the mucosa than in those adjacent to the intestinal lumen. By the 24th hour the concentration of the isotope was strikingly higher and more uniform from cell to cell. The mucus in the intestinal lumen was also highly radioactive. At the end of 48 hours very little of the sulfur-35 remained in the intestinal wall or could be made out in the mucus of the lumen: the autoradiographic reaction was faint and diffuse as contrasted with the punctiform and intense reaction given by the specimens removed at the end of shorter intervals of time.

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EXPLANATION OF PLATES

PLATE 7

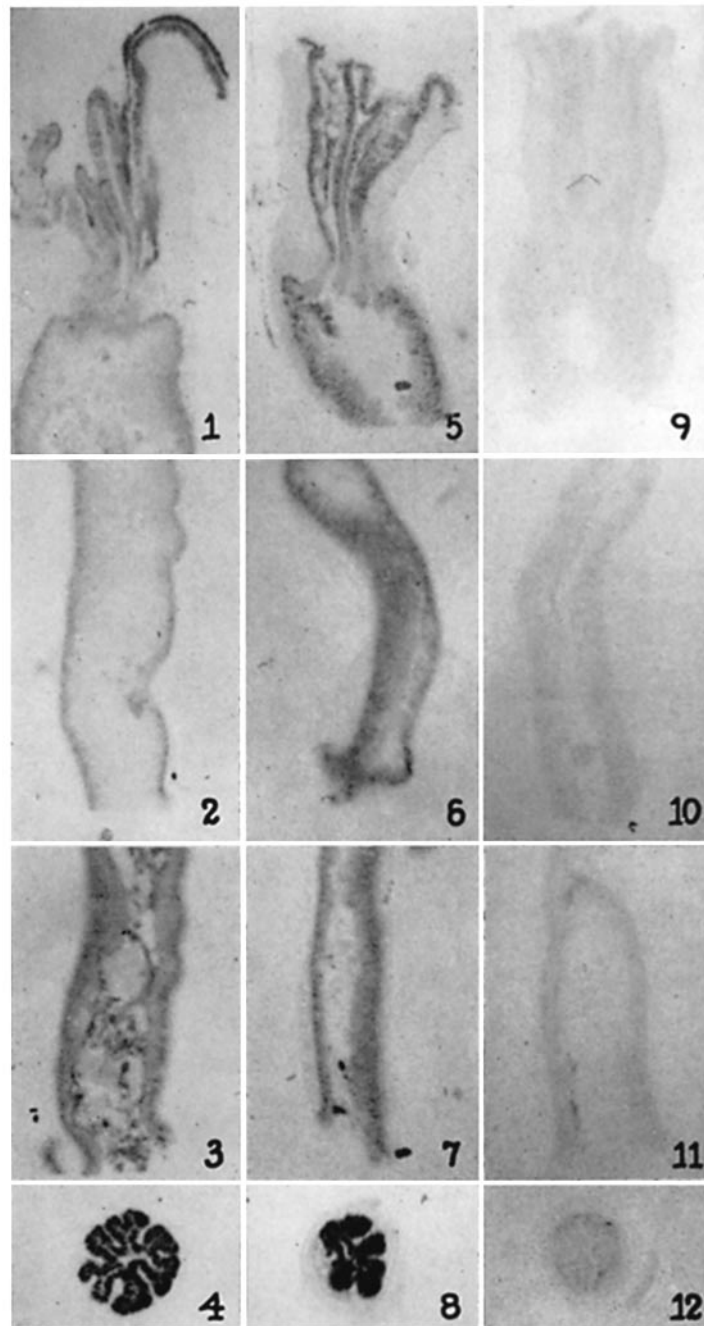
Contact autoradiograms of sections from segments of the gastrointestinal tracts removed from rats at the end of intervals of time after the intraperitoneal injection of S³⁵-sulfate. A comparison of the autoradiograms, from left to right, shows that the fixation of S³⁵-sulfate into compounds which are insoluble in aqueous formalin, was rapid at all levels of the tract and that these materials were also rapidly removed so that by the 48th hour only a faint autoradiographic reaction was seen. The extent to which the labelled sulfate was fixed at different levels of the intestinal tract can be seen by comparing the pictures from top to bottom. × 5.

FIGS. 1, 5, and 9. Autoradiograms of longitudinal sections of the pyloric region of the stomach and the duodenum subjacent to it.

FIGS. 2, 6, and 10. Autoradiograms of longitudinal sections of the duodenum.

FIGS. 3, 7, and 11. Autoradiograms of longitudinal sections of segments removed from the middle of the intestine.

FIGS. 4, 8, and 12. Autoradiograms of transverse sections of the colon.



6 hours

24 hours

48 hours

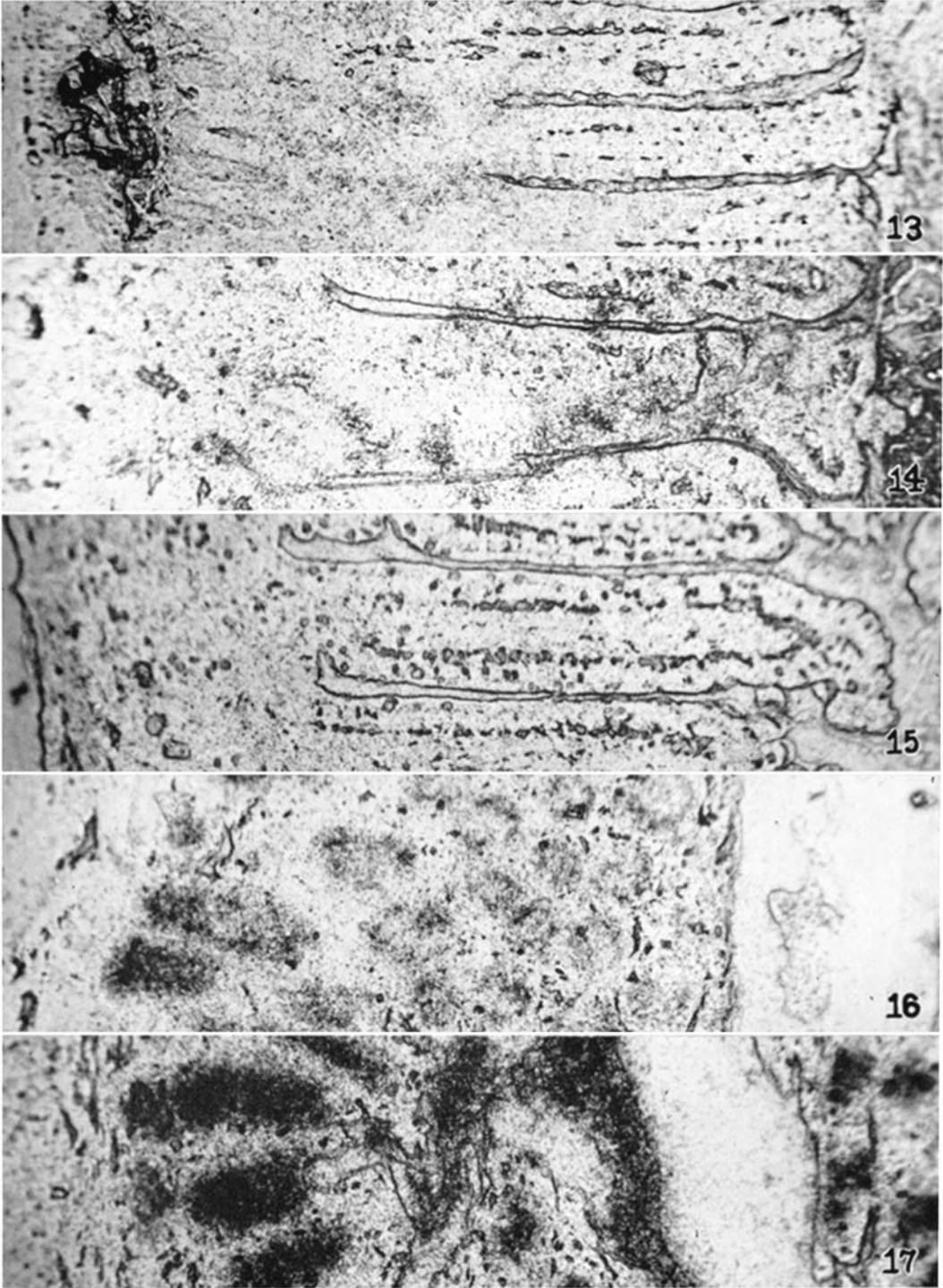
(Dziewiatkowski: S³⁵-sulfate turnover in mucosa)

PLATE 8

Coated autoradiograms of sections from the intestinal tracts of rats at the end of intervals of time after the intraperitoneal injection of S^{35} -sulfate. $\times 186$.

FIGS. 13, 14, and 15. Autoradiograms of longitudinal sections of the duodenum removed 6, 24, and 48 hours, respectively, after the injection of the labelled sulfate. At the end of the 6 hour interval the highest concentration of the isotope is seen associated with the cells deepest in the crypts of the mucosa; whereas, at 24 hours the goblet cells closest to the intestinal lumen have the highest concentration of the sulfur-35. At this time the mucus in the intestinal lumen is also labelled. By the 48th hour the intestinal wall contains only a small amount of diffusely distributed sulfur-35.

FIGS. 16 and 17. Autoradiograms of longitudinal sections of the colon removed 6 and 24 hours, respectively, after the injection of labelled sulfate. Again at the end of the 6 hour interval the highest concentration of sulfur-35 can be seen in the goblet cells located most deeply in the glands of Lieberkühn. A much higher and nearly uniform concentration of the isotope is seen in the goblet cells after 24 hours at which time it is also apparent that the mucus in the lumen is highly radioactive.



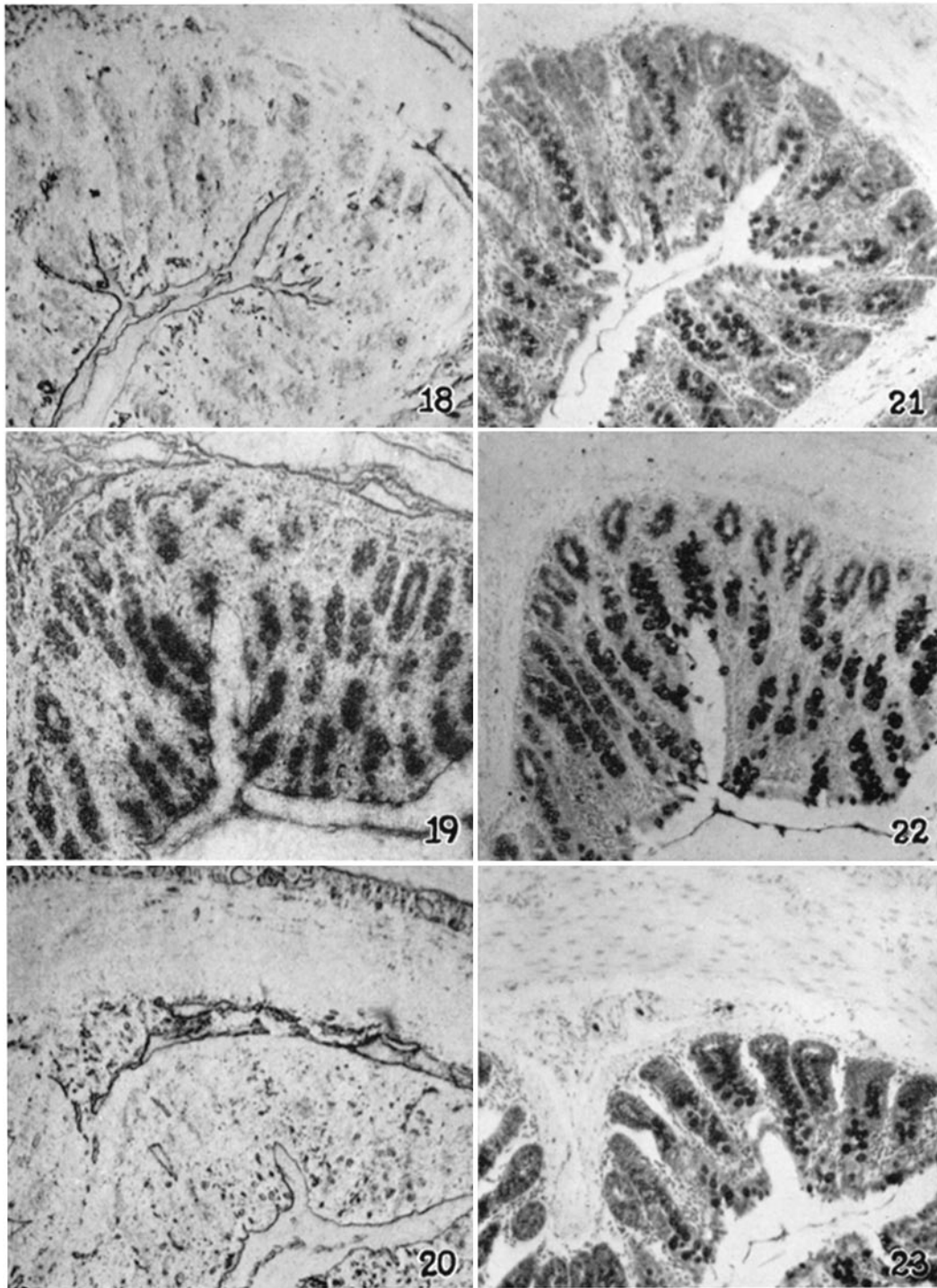
(Dziewiatkowski: S³⁵-sulfate turnover in mucosa)

PLATE 9

Coated autoradiograms of transverse sections of the colon of rats at the end of intervals of time after the intraperitoneal injection of S^{35} -sulfate. Photomicrographs of comparable regions in adjacent stained sections show that the distribution of the goblet cells corresponds well to the observed autoradiographic reaction at the end of the 6th and 24th hour interval.

FIGS. 18, 19, and 20. Autoradiograms of transverse sections of the colon removed 6, 24, and 48 hours respectively, after the injection of labelled sulfate. Evidence of the passage of the S^{35} -sulfate from the mucous cells deepest in the glands of Lieberkühn to those adjacent to the intestinal lumen can be seen. That this is a rapid process is again affirmed by the fact that by the end of the 48th hour interval the autoradiographic reaction is faint and diffuse.

FIGS. 21, 22, and 23 are photomicrographs of stained sections adjacent to those which produced the autoradiograms reproduced as Figs. 18 to 20, respectively. The regions shown are comparable. Toluidine blue, 0.1 per cent in 30 per cent ethanol was used to stain the sections. The goblet cells of the mucosa and the mucus in the intestinal lumen stained metachromatically, whereas, the rest of the intestinal tissue was tinted a light blue; the mucosa because of its greater over-all binding of the dye stands out in contrast to the other tissue layers of the intestine.



(Dziewiatkowski: S^{35} -sulfate turnover in mucosa)