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# Specificity of Indium-111 Granulocyte Scanning and Fecal Excretion Measurement in Inflammatory Bowel Disease—An Autoradiographic Study

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*The validity of $^{111}In$ granulocyte scanning and fecal excretion measurement, as a reflection of loss of cells into the gastrointestinal tract, was studied using an autoradiographic technique in 11 patients in whom $^{111}In$ granulocyte scan and colonoscopy were carried out simultaneously. $^{111}In$ granulocytes were injected 1.5–4 hr prior to colonoscopy, and intraluminal fluid, mucosal brushings, and colonic biopsies were collected during the colonoscopy. In two patients with no histological evidence of inflammatory bowel disease, and four patients with clinically and histologically inactive inflammatory bowel disease, no $^{111}$Indium was detected in fluid, brushing, or biopsies. In five patients with active disease, 85% of the $^{111}In$ activity in colonic fluid was precipitated by low-speed centrifugation. Autoradiography confirmed that the label remained attached to whole granulocytes in colonic fluid and mucosal brushings. Studies on biopsies, at intervals up to 4½ hr following labeled granulocyte injection, demonstrated labeled polymorphonuclear neutrophils (PMNs) on the inflamed epithelial surface, with occasional cells in crypt abscesses by 110 min. We conclude that the techniques of $^{111}In$ granulocyte scanning and fecal counting in patients with IBD are specifically measuring cell loss; labeled PMNs are capable of migrating through the gastrointestinal mucosa, in active disease, within 2 hr of administration.*

Indium-111 granulocyte scanning is now a well-established technique for diagnosis and assessment of inflammatory bowel disease (IBD) (1–3). Indium-111 is used to label granulocytes *in vitro*, and then cells are subsequently injected intravenously. The isotope localizes in inflamed bowel and subsequently can be counted in feces. The validity of the test in distinguishing cell loss from protein-losing enteropathy critically depends on *in vivo* stability of granulocyte labeling. We have therefore investigated this, using an autoradiographic technique, in 11 patients in whom $^{111}$In granulocyte scans and colonoscopy were carried out simultaneously.

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## MATERIALS AND METHODS

Eleven patients were investigated as detailed later. $^{111}$In granulocyte scan and colonoscopy were carried out simultaneously either for investigation of diarrhea or for assessment of activity and distribution of known inflam-



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matory bowel disease. All patients gave informed consent. Indium-111 granulocyte scans were performed as described previously (4, 5). In brief, pure granulocytes were obtained from patients' whole blood using the sedimentation and double-density gradient method. Pure granulocytes were labeled with $^{111}$In tropolonate in autologous plasma. Then 200–300 μCi of labeled granulocytes in 10 ml plasma were injected intravenously and abdominal scans performed using a medium-energy parallel-hole collimator 40 min and 4 and 24 hr following injection. The effective dose equivalent of radiation received from the administered activity of 200 μCi of $^{111}$In is 0.5 Rem (2.4 Rem/mCi) (12). Colonoscopy was carried out with GIFITL Olympus colonoscope 1½–4 hr following injection of labeled granulocytes. Each patient was given pethidine 50 mg and midazolam hydrochloride 2.5–5 mg intravenously just prior to colonoscopy. At colonoscopy, samples of intraluminal fluid, mucosal brushings using a nylon cytology brush, and biopsies were taken and processed as detailed below.

**Autoradiographic Techniques.** Both smears and tissue sections were examined using the stripping film radiographic technique (7), using methanol-fixed smears and formol–alcohol-fixed biopsies. Kodak AR10 emulsion was stripped and floated from plates and overlaid on smears or sections mounted on chrome gelatinized slides. Slides were developed after 24 hr and subsequently hematoxylin and eosin stained.

## RESULTS

Of the 11 patients, six had no evidence of active inflammation in the colon, histologically or endoscopically. This included two patients investigated for diarrhea in whom no cause was found, two patients with diagnosis of ulcerative colitis, and two patients with diagnosis of Crohn's disease previously established histologically. The absence of active inflammatory bowel disease on histology or endoscopy was confirmed by negative $^{111}$In granulocyte scans in all these six patients. The remaining five patients had active colitis on colonoscopic and histological evidence and $^{111}$In granulocyte scans. Four of these patients had ulcerative colitis (UC) and one Crohn's colitis (CD). The distribution of colitis was left-sided in three (all histologically ulcerative colitis) and pancolitis in two (one UC, one CD).

In the six patients without evidence of acute inflammation in the colon, colonic fluid, mucosal brushings, and biopsies were negative on autoradiography, with no difference in the grain counts seen in emulsion laid on background and that overlying the specimens. In contrast, autoradiography in all five patients with active inflammatory bowel disease showed radioactivity concentrated over whole granulocytes with negligible background activity in the colonic fluid (Figure 1) and mucosal brushings (Figure 2). In the colonic fluid, 85% ± 3 (SD) of the $^{111}$In counts were precipitated by 2000*g* centrifugation for 5 min. Serial studies on colonic biopsies taken from sites of inflammation identified endoscopically, at intervals up to 4½ hr following labeled granulocyte injection, demonstrated labeled granulocytes on the inflamed epithelial surface (Figure 3), with occasional granulocytes in crypt abscess (Figure 4) by 110 min. The inflammatory involvement in these areas was confirmed both by histological examination, and by the appearances of the abdominal scans at 40 min and 4 and 24 hr after injection.

## DISCUSSION

Objective assessment of disease activity in Crohn's disease is an important means of managing individual patients and also monitoring treatment in clinical trials. The ideal measurement for the assessment of disease activity in inflammatory bowel disease has been discussed (8). It should be easy to perform, respond quickly to changes in disease activity, and be specific for gastrointestinal inflammation. Serological tests, although easy to perform and frequently responding rapidly, all suffer from the disadvantage of not being specific for gastrointestinal inflammation and therefore are easily influenced by intercurrent events. A measurement of protein loss into the gut has been suggested as the most sensitive means of assessing gastrointestinal inflammation (9). It is technically demanding to perform, in a manner analogous to indium scanning, but it responds rapidly to changes in disease activity and specifically reflects abnormalities in the gastrointestinal tract. It has been shown to correlate well with disease activity in series of patients with inflammatory bowel disease (9). However, although protein-losing enteropathy occurs in the setting of inflammatory bowel disease, protein loss can occur under many conditions, some of which are not associated with inflammation.

Quantitative fecal $^{111}$In granulocyte excretion apparently fulfills the criteria for rapid response to changes in disease activity and specificity for gastrointestinal inflammation. Its technical complexity is similar to that of protein loss studies and $^{111}$In excretion was shown to correlate well with disease activity in Crohn's disease (3). In a small group of patients in whom fecal protein loss and fecal gran-

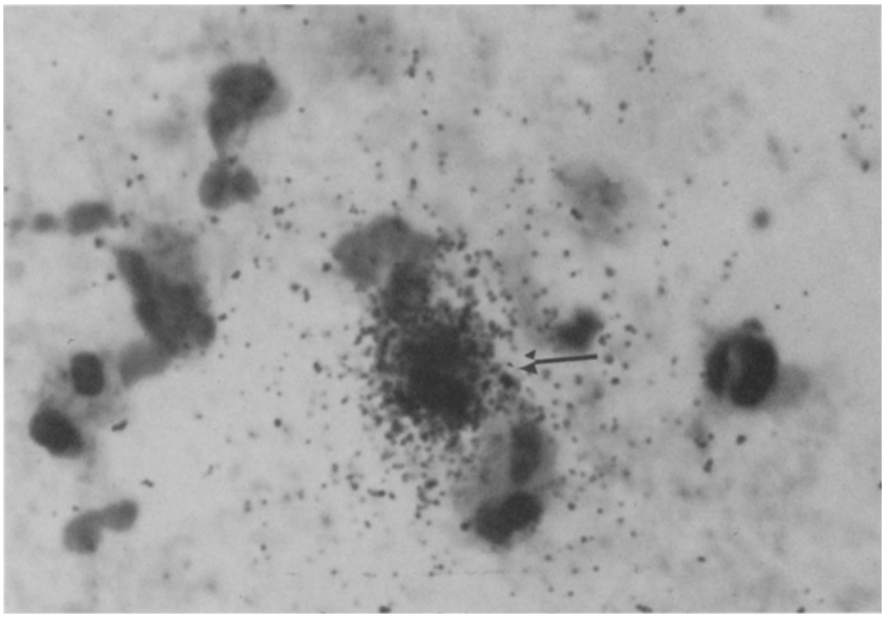

**Fig 1.** Labeled granulocyte in the colonic fluid smear (×1800) of a patient with active ulcerative colitis.

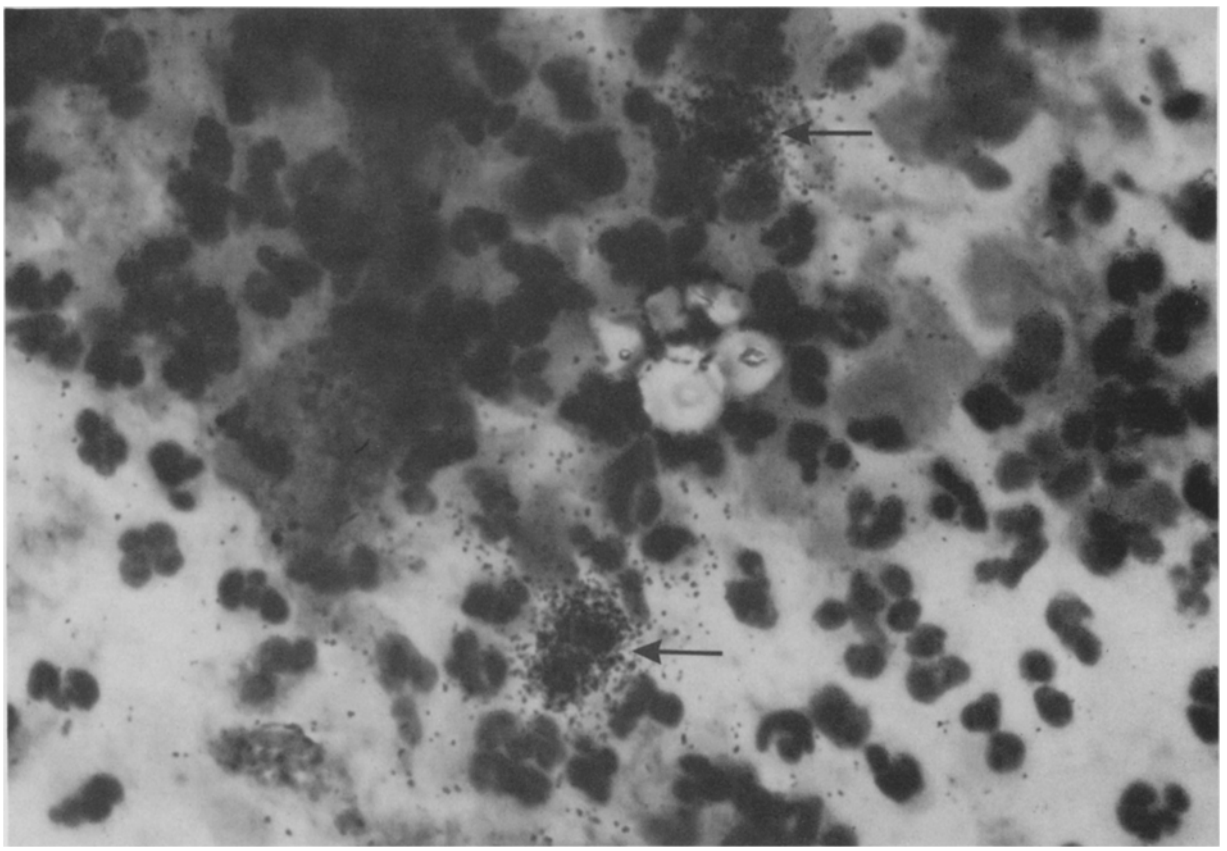

**Fig 2.** Labeled granulocytes in the mucosal brushing smear (×1800) of a patient with ulcerative colitis.

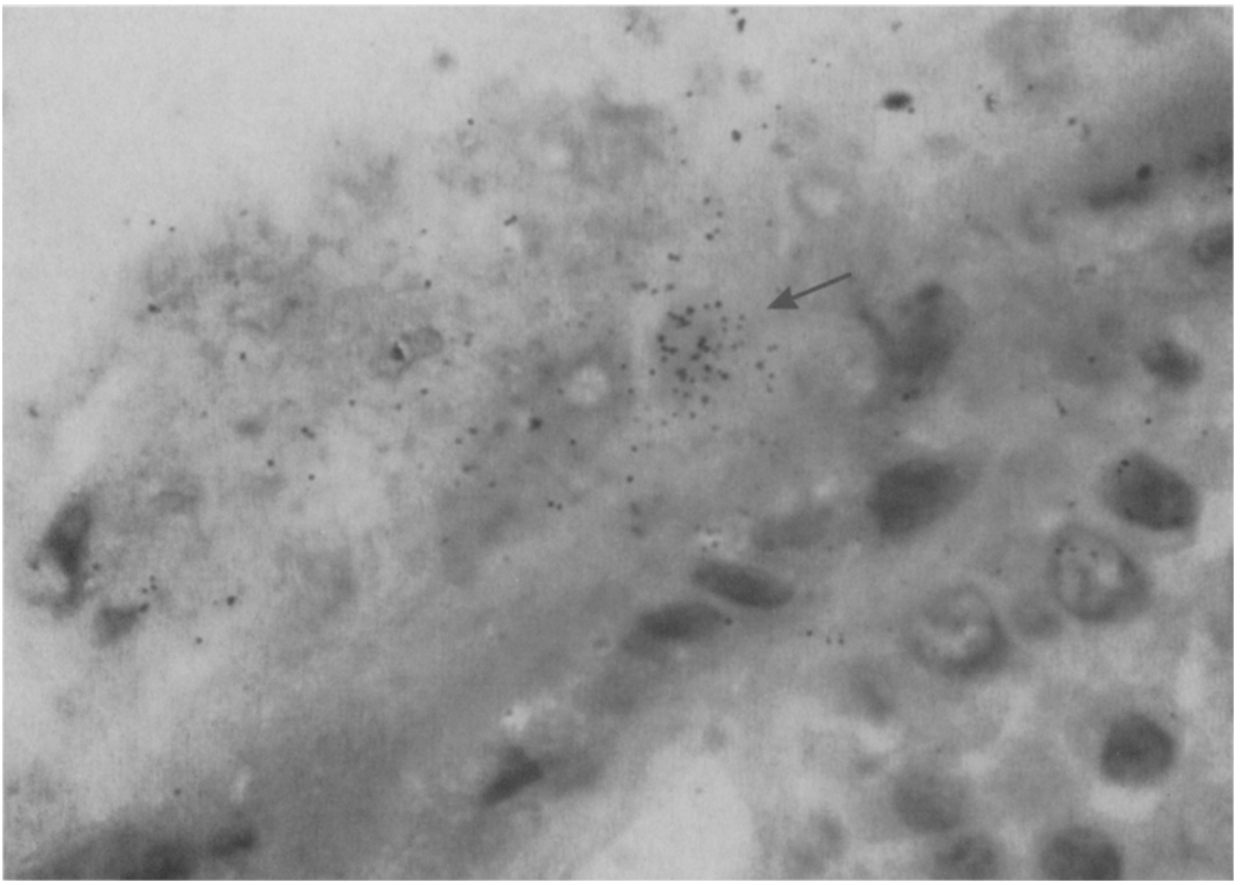

**Fig 3.** Labeled granulocytes on the inflamed epithelial surface (×1800), from colonic biopsy of a patient with active ulcerative colitis.

ulocyte excretion were simultaneously assessed, there was a significant correlation between the two (3). However, interpretation of fecal loss of $^{111}$In as a reflection of gastrointestinal inflammation after injection of $^{111}$In-labeled autologous leukocytes is critically dependent on the stable labeling of indium to the granulocytes. Free indium will label transferrin *in vivo*, and indium-labeled transferrin has in fact been used as an estimation of protein loss in patients with protein-losing enteropathy in the absence of apparent inflammatory lesions in the gut (10).

Currently indirect *in vivo* and *in vitro* evidence has suggested that there is stable labeling of $^{111}$In to granulocytes. Thus indium is avidly bound intracellularly (11), and measurements of *in vitro* and *in vivo* elution of indium have not suggested that this is a major problem (12, 13). However this paper presents direct evidence of stable labeling of $^{111}$In to granulocytes, showing attachment of indium to the cell throughout its migration to the bowel lumen. Thus indium fecal counting is a valid technique for assessing fecal granulocyte loss and represents a specific means of quantitating the inflammatory component in inflammatory diseases of the bowel. Furthermore, the lack of reabsorption of indium (14) indicates that fecal measurements give objective measurements of polymorphonuclear granulocyte excretion into the intestine at whatever level this occurs.

We have also demonstrated directly the rapidity of the transport of labeled granulocytes through the inflamed bowel wall, appearing within the lumen within 4 hr. This indicates that this technique can be used to study *in vivo* granulocyte dynamics in a variety of inflammatory processes.

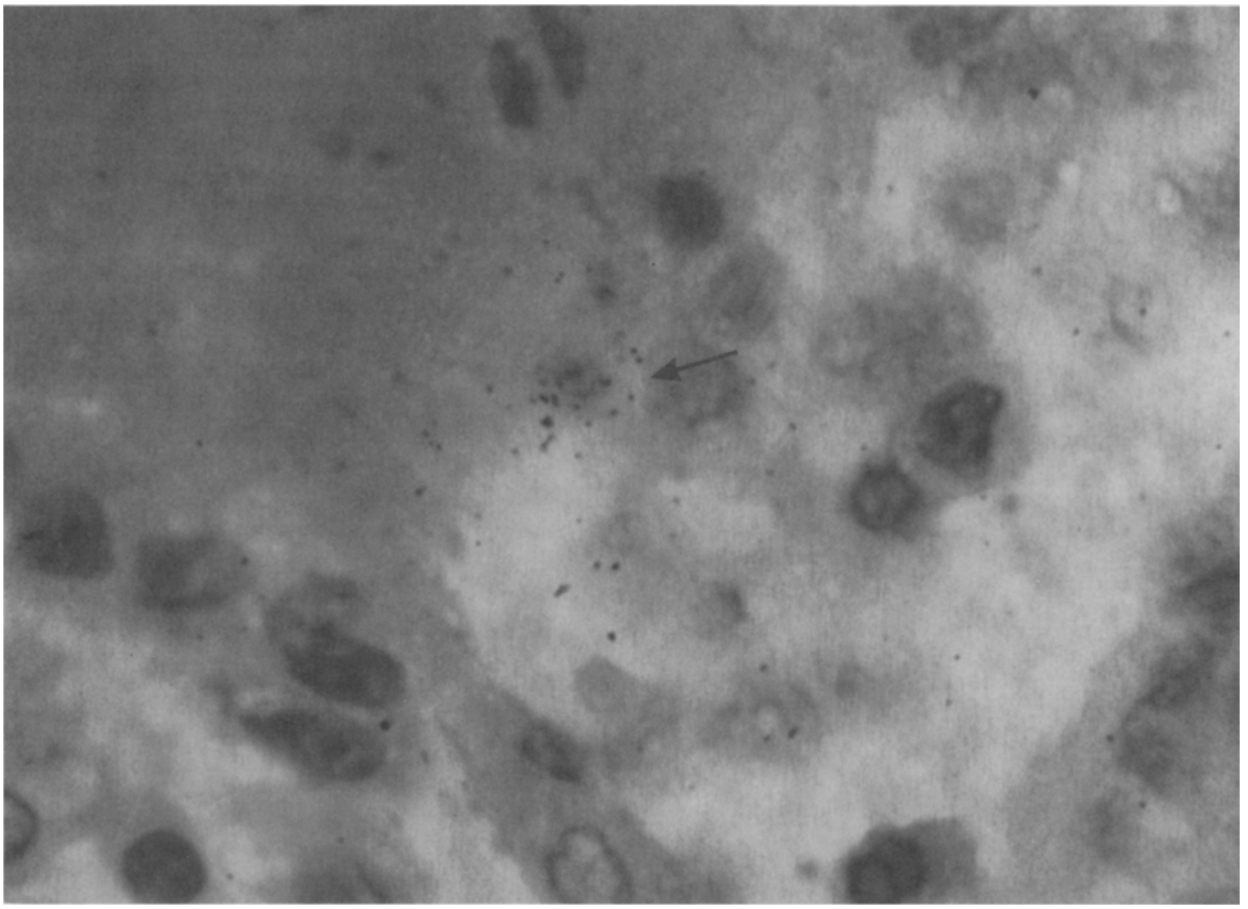

**Fig 4.** Labeled granulocyte in crypt abscess (×1800) from colonic biopsy of a patient with active ulcerative colitis.

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## REFERENCES

1. Saverymuttu SH, Peters AM, Hodgson HJF, Chadwick VS, Lavender JP: Indium-111 autologous leukocyte scanning: Comparison with radiology for imaging the colon in inflammatory bowel disease. Br Med J 285:255–257, 1982
2. Saverymuttu SH, Peters AM, Hodgson HJF, Chadwick VS, Lavender JP: 111-Indium leukocyte scanning in small bowel Crohn's disease. Gastrointest Radiol 8:157–161, 1983
3. Saverymuttu SH, Peters AM, Lavender JP, Pepys MB, Hodgson HJF, Chadwick VS: Quantitative fecal indium-111-labeled leukocyte excretion in the assessment of disease in Crohn's disease. Gastroenterology 85:1333–1339, 1983
4. Danpure HJ, Osman S, Brady F: The labeling of blood cells in plasma with 111-In tropolonate. Br J Radiol 55:247–249, 1982
5. Saverymuttu SH, Peters AM, Danpure HJ, Reavy HJ, Osman S, Lavender JP: Lung transit of 111-indium-labeled granulocytes. Scand J Haematol 30:151–160, 1983
6. Johansson L, Mattsson S, Knosslin B: Effective dose equivalent from radiopharmaceuticals. Eur J Nucl Med 9:485–489, 1984
7. Roger AW: Techniques of Autoradiography. Amsterdam, Elsevier, 1975
8. Hodgson HJF: Methods in clinical pharmacology: Assessment of drug treatment in inflammatory bowel disease. Br J Clin Pharmacol 14:159–170, 1982
9. Kaufman S, Chalmer B, Heilman R, Beeken W: A prospective study of the course of Crohn's disease. Dig Dis Sci 24:269–276, 1979
10. Saverymuttu SH, Peters AM, Lavender JP, Hodgson HJF: $^{111}$Indium transferrin scanning in the detection of protein losing enteropathy. Eur J Nucl Med 8:40–41, 1983