Permeability of the rectosigmoid mucosa to tritiated water in normal subjects and in patients with mild idiopathic ulcerative colitis¹

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EDITORIAL COMMENT Absorption of water from the colonic mucosa is impaired in patients with proctocolitis even when this disease is mild.

It is well known that one severely disabling manifestation of ulcerative colitis is diarrhoea, and among the mechanisms suggested to account for it in this condition has been a derangement of absorption across the colonic mucosa.

Previous studies utilizing other substances than water have failed to demonstrate deranged absorption from the inflamed rectosigmoid of patients with idiopathic ulcerative colitis (Levitan, Bikerman, Burrows, and Ingelfinger, 1963). The only quantitative information concerning absorption of water from the diseased colon in ulcerative colitis was obtained from studies on anaesthetized patients with advanced disease who were about to undergo colectomy (Duthie, Watts, de Dombal, and Goligher, 1964). In addition, these patients were treated with adrenal hormones. It was felt, therefore, that it would be of interest to obtain information about the transfer of water across the inflamed rectosigmoid mucosa in patients who had untreated mild ulcerative colitis in remission and were not under the influence of anaesthetics.

The aim of the present study was to measure the permeability of the rectosigmoid mucosa to tritiated water in normal volunteers and in patients with untreated ulcerative colitis. The appearance of the tritiated water in venous blood following rectal instillation was used as a measure of colonic permeability. No attempt was made to measure net water absorption. This relatively simple technique was utilized in the hope that useful information could be obtained without physiological disturbance and without subjecting the patients to long, tedious intubation procedures.

METHODS

The normal subjects were healthy volunteers aged 21 to 55. The patients in the same age range had mild idiopathic ulcerative colitis involving the distal rectosigmoid as judged by sigmoidoscopy and barium enema, but did not have diarrhoea when studied. All subjects were well hydrated, afebrile, and were not receiving any specific therapy at the time of the studies. Before testing the rectosigmoid permeability to tritiated water, preliminary studies were carried out on the normal subjects and on the patients with ulcerative colitis to determine total body water by a dilution technique (using intravenously administered tritiated water) and the rate of disappearance of intravenously administered tritiated water from venous blood.

The results showed that the total body water was within normal limits in all of the patients studied ($60 \pm 10\%$ of body weight) and that the disappearance rate of tritiated water from venous blood after intravenous injection was also within normal limits in both controls and in patients.

Before the rectal studies, all subjects were sigmoidoscoped after spontaneous bowel evacuation. The rectosigmoid area was cleaned with swabs and inspected carefully. After this preparation, a test solution consisting of 40 ml. of 0.85% sodium chloride containing 0.5 mc. of tritiated water was injected into the rectosigmoid via rectal tube. This volume of test solution was chosen on the basis of previous results in normal subjects which showed that such an amount of material could be retained in the rectosigmoid by most persons without causing discomfort or reflux into the proximal colon (Levitan *et al.*, 1963). In the present investigation fluoroscopic observations made over a period of three hours in patients with mild ulcerative colitis showed that a test solution of 0.85% sodium chloride containing barium remained for this period of time in the rectosigmoid of seven out of 10 patients. In the normal control subjects the solution was also observed to remain in the rectosigmoid in most instances, that is, in eight out of 10 studies.

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In an attempt to evaluate the possibility that the rectally instilled tritiated water in the test solution may become more diluted in the patients than in the controls, the following additional preliminary experiments were undertaken. A water-soluble, non-absorbable marker, P.E.G. (polyethylene glycol, mean molecular weight of 4,000) in the concentration of 1% was added to the 40 ml. of 0.85% Na Cl containing 0.5 mc. of tritiated water. This material was then instilled rectally as previously described into five normal subjects and five patients with mild ulcerative colitis. The test solution was sampled at five-minute intervals for 15 minutes after rectal instillation and it was analyzed for P.E.G. concentration and specific activity of tritiated water as previously described (Levitan, Fordtran, Burrows, and Ingelfinger, 1962). The results did not show evidence for significant dilution of the non-absorbable marker or tritiated water by fluid coming from the proximal colon, either in controls or patients.

In the rectal permeability studies the subjects remained recumbent after rectal injection of the test solution and were instructed to retain this material. Only subjects who retained the test solution for at least three hours were included in this study. Five ml. samples of venous blood were withdrawn via an indwelling venous catheter at three, five, 10, 15, 30, 45, 60, 90, 120, and 150 minutes respectively. Precautions were taken to avoid venous stasis. The blood was put in tubes containing a drop of heparin. Specimens were centrifuged and the plasma was removed without causing haemolysis. The amount of tracer in the plasma was determined by counting samples in a Tricarb liquid scintillation counter. Results were expressed as the percentage of rectally administered tracer present per litre of plasma. These percentages were plotted as a function of time in each experiment. The curves were analyzed mathematically with the aid of a digital computer (see Appendix) to determine: (1) the appearance rate of the tracer at different times $\frac{dx}{dt}$; (2) the actual percentage of rectally administered tracer in the plasma at its maximum and at 60 minutes; (3) the time at which the maximum was reached.

The results of the studies on patients were compared to those on normal controls.

RESULTS

All studies in normal subjects as well as in patients with ulcerative colitis revealed that tritiated water appeared in the venous blood rapidly (Fig. 1). Noticeable amounts of tracer were present at three minutes. The tritium appearance curves reached a plateau between 40 and 60 minutes.

In Fig. 1 are plotted the mean values and $\pm$ S.E.M. (standard error of the mean) for the appearance rate of tritiated water in venous blood in 10 control subjects and in five patients with ulcerative colitis.

The differential $\frac{dx}{dt}$ at five minutes was 0.12 ± 0.03 for the controls and 0.056 ± 0.001 for the patients, a significant difference between the two groups ($P =$

FIG. 1. Appearance rate of tritiated water in venous blood after rectal instillation of 40 ml. 0.85% Na Cl containing 0.5 mc. of H_2^3O .

The concentrations of tritiated water in plasma, expressed as a percentage of the amount of tracer given rectally, are plotted against time on a semilogarithmic scale.

The dots are mean values ± 1 S.E.M. (standard error of the mean). The data presented are for 10 normal subjects and five subjects with ulcerative colitis (six tests).

0.01). The mean time $\pm$ S.E.M. at which the curves reached a peak was 52.6 ± 9.5 minutes in the controls and 80.8 ± 6.7 minutes in the patients with ulcerative colitis. This difference is statistically significant ($P = 0.04$). The area under the curve t_0 to t_{max} was 90 ± 13 for the controls and 110 ± 13 for the patients with ulcerative colitis, a difference considered not to be statistically significant.

The area under the curve at 60 minutes was 117.6 ± 28 in controls and 75 ± 16.5 in ulcerative colitis patients. This difference is significant for $P = 0.05$.

REPRODUCIBILITY

In three normal subjects the appearance rate of tritiated water in the venous blood was determined

on two separate occasions. One patient with ulcerative colitis was also studied twice, once during exacerbation and again six months later during remission. The blood curves appeared similar (Figs. 2 and 3).

DISCUSSION

Inasmuch as the values obtained for total body water and for the rate of disappearance of intravenously administered tritiated water from venous blood did

FIG. 2. Reproducibility of appearance of H_2^3O in venous blood after rectal instillation of 40 ml. 0.85% NaCl containing 0.5 mc. of H_2^3O . The results of the first study are represented by dots connected by a solid line and those of the repeat study obtained on a separate day by crosses connected by a dotted line.

The concentrations of the tritiated water in the plasma, expressed as a percentage of amount of tracer given rectally, are plotted against time on a semi-logarithmic scale.

FIG. 3. Reproducibility of H_2^3O appearance in venous blood after rectal instillation of 0.85% NaCl containing 0.5 mc. of H_2^3O in a patient with idiopathic ulcerative colitis involving the descending and sigmoid colon as well as rectum. The results obtained during exacerbation are represented by dots connected by a solid line, and those during remission by crosses connected by a dotted line. The concentrations of the tritiated water in the plasma are plotted against time on a semi-logarithmic scale.

not differ in normal subjects and in patients studied, and since the specific activity of the tritium in the test solution did not change differently in the patients than in the controls, the venous appearance curves of labelled water following rectal instillation of tritiated water can provide meaningful information concerning rectosigmoid permeability. We would like to emphasize that the patients we studied had no diarrhoea during the time of the experiments. It is very likely that the technique that we employed would not be suitable for the study of subjects with diarrhoea since the specific activity of the test solution could be diluted differently in them than in the controls. Our experimental design does not allow us precisely to normalize the data from the controls and the patients to a standard surface area of the colon. However, other techniques used in living subjects for the study of absorption from the bowel have similar limitations (Gray and Ingelfinger, 1966).

It is logical to assume that the tracer appeared in venous blood following passage across the rectosigmoid mucosa. During this transfer, part of the labelled water diffused continuously out of the blood into extravascular compartments. A small part of the tracer may have diffused back into the bowel lumen or remained in the wall of the colon. Previous studies, however, have shown that less than 3% of the tracer could be recovered in the entire colonic lumen following intravenous administration of tritiated water in normal subjects (Levitan *et al.*,

1962). The venous blood appearance curves of the tritiated water were, therefore, the results of two principal events: (1) diffusion of the tracer from the colonic lumen into the blood; (2) diffusion of the tracer out of the blood. In the studies reported here, the appearance curves do not reflect net absorption of water, but are an expression of rectosigmoid permeability. They primarily reflect the flux of tritiated water from the colonic lumen into venous blood.

Despite certain limitations in the technique used, easily obtained information can be provided concerning rectosigmoid permeability without subjecting patients to prolonged, tedious colonic intubation procedures (Levitan *et al.*, 1962), required for precise measurements of colonic absorption. Our data show that the appearance rate of tritiated water into venous blood following its rectal instillation is a relatively reproducible phenomenon in the same subject.

Tritiated water appeared more rapidly in the blood of normal subjects than in patients with ulcerative colitis, the point of maximum concentration also occurring more quickly in the normals. The integral of the appearance curve is a reflection of the amount of tracer present in the plasma. This value at the time of the peak of the curves was similar in controls and in patients.

Our data show that even in patients with mild ulcerative colitis, the transfer of water across the colonic mucosa was slower, but eventually as much

tracer was transferred to the plasma of the patients as to that of normal controls. The apparently slower transfer rate of tracer across the colonic mucosa in patients with a normal total body water can be explained at the very least in the following ways: (a) that less mucosa was exposed to the test solution in these patients, that is, less mucosa being available due to destruction or fluid being distributed over a small area; (b) that the mucosa was less permeable in patients than in controls; (c) that colonic mucosal blood flow was decreased in patients; (d) that the specific activity of the test solutions was altered differently in the two groups after rectal instillation, that is, more dilutions occurring in the patients than in the controls although this seems to be unlikely in view of our preliminary studies.

While the results of our experiments show a delayed transfer of tritium across the colon in patients with mild ulcerative colitis, no conclusions can be established concerning the actual mechanism by which this abnormality occurs.

During laparotomy, just before colectomy, Duthie *et al.* (1964) studied net water absorption and unidirectional water fluxes in diseased isolated colonic loops of anaesthetized patients with ulcerative colitis. The patients had been treated with adrenal cortical hormones. They found a significant decrease in net water absorption from the inflamed colonic loops when compared with the values obtained from control subjects. This decrease in net water absorption was considered to result primarily from a decrease in the luminofugal flux (flux out of the colonic lumen-insorption) (Duthie *et al.*, 1964).

While it is not valid to extrapolate from the rate of appearance of tritium in the venous blood to net movement of water out of the colon, we feel that it is permissible to compare the venous appearance curves of tritiated water at least in a qualitative fashion to the luminofugal water flux (flux out of the colon). It is interesting to note that Duthie *et al.* (1964) found a decrease in the water flux out of the colon as we did in our studies.

It should be mentioned that in previous studies, utilizing molecules larger than water, no derangement of rectosigmoid permeability in ulcerative colitis was found (Levitan *et al.*, 1963). In these experiments, phenolsulphonphthalein radio-iodine and sulphisoxazole diethanolamine were used.

SUMMARY

The appearance rate of tritiated water in venous blood following rectal instillation was studied in

normal subjects and in patients with mild ulcerative colitis in remission. All of the patients studied had normal total body water and were afebrile. Venous blood appearance curves were reproducible in three normal subjects and in one patient with ulcerative colitis who were studied on different days. Following rectal injection the labelled water appeared in the venous blood in three to five minutes and the tracer concentration in the plasma reached a plateau in 40 to 60 minutes.

In patients with idiopathic ulcerative colitis, the rate of appearance of tracer in the blood was slower. The mean time at which the curves reached a peak was 52.6 minutes in the controls as compared with 80.8 minutes in the patients with ulcerative colitis. These findings suggest that water transfer across the inflamed rectosigmoid is impaired even in patients with mild ulcerative colitis.

APPENDIX

With the help of the digital computer several forms of functions were tried out to fit our data:

$$A \ y(t) = A e^{\alpha t}$$

$$B \ y(t) = A e^{\beta t^2 + \alpha t}$$

$$C \ y(t) = A t e^{\beta t^2 + \alpha t}$$

$$D \ y(t) = a + bt + ct^2 + dt^3$$

Using the least square method the polynomial formula D (with four independent coefficients) described our data best. The addition of a fifth coefficient did not improve the fit significantly.

The following calculations were done for each study:

1 The rates of appearance of tritiated water were calculated by the differential of the polynomial D: $y'(t) = b + 2ct + 3dt^2$

2 The total amount of tracer in the blood by calculating the integral

$$\int_0^t y(t) dt = at + 1/2 bt^2 + 1/3 ct^3 + 1/4 dt^4$$

3 The time at which the curve reached its peak was solved by $y' (= t_{max}) = 0$.

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