THE OCCURRENCE OF SKATOL IN THE HUMAN INTESTINE.

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(Received for publication, December 7, 1907.)

The color reaction between paradimethylaminobenzaldehyde (Ehrlich's aldehyde) and skatol\(^1\) gives us an easy method of detecting the presence and of estimating the quantity of this putrefactive product. The employment of the method in routine and experimental work upon the digestive tract has led to the accumulation of numerous observations that have an interest both for the physiologist and the physician. It is my wish to bring together here the chief results of these observations.

It seems to be a general impression, at least among writers of text-books of physiological chemistry, that skatol is a regular product of intestinal putrefaction in the feces. Probably this impression is due to the statement made by Brieger to this effect\(^2\)—a statement which requires some modification. Among healthy children under ten years of age I have found the occurrence of skatol in the feces to be quite exceptional whether they live on a milk diet or on a mixed diet inclusive of meat. Among adults also there are many who show no trace of skatol. It is, however, true that one frequently finds a trace of skatol in the freshly voided feces of healthy individuals.

Where there is an excessive degree of intestinal putrefaction there may be a marked increase in the skatol content of the feces. Whereas in persons who are normal (in the sense that they are

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\(^1\) On the Separation of Indol from Skatol and their Quantitative Determination, this Journal, ii, p. 267, 1906.

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unconscious of any disorder of digestion) one rarely finds more than 0.5 milligram of skatol in 100 grams of fresh feces, the quantity may reach 8 or 10 milligrams in persons who are the subjects of disturbed intestinal digestion. These quantities I have seldom observed and it may be safely stated that it is rare to find more than 5 milligrams in 100 grams, even where putrefactive decomposition is intense. In this respect the skatol content of the lower intestine differs from the indol content, which is sometimes greater than is represented by the above figures. Moreover it is true that among apparently normal individuals it is more common to find traces of indol in the feces than traces of skatol. To this rule there is observed an occasional exception in which skatol can be detected but not indol.

The detection of skatol in the feces in the absence of indol of course does not prove that there has been no production of indol in the gut, since it may have been formed at some level above the rectum and subsequently absorbed entirely. It is clear that this might be the case where we find skatol but no indol, despite the fact that indican is present in the urine and cannot be ascribed to any but an intestinal origin. The presence of skatol without indol in the lower part of the bowel might be ascribed either to a more ready absorption of indol than of skatol (assuming them to be produced in equal abundance at the same level), or to a relatively late production of skatol.

The following experiment was made with a view to determining whether the absorption of indol from the intestine is more rapid than that of skatol.

Into a loop of dog's ileum which had been previously washed out with salt solution there was introduced 100 cc. of a solution prepared by dissolving 5 mg. of indol and 5 mg. of skatol in 2 cc. of alcohol and diluting to 250 cc. with physiological salt solution. After the introduction of this fluid, the gut was returned into the abdominal cavity. The animal was killed at the end of 35 minutes, when the gut had in a large degree emptied itself. 26 cc. of fluid were found in the gut. After filtration this fluid was distilled and the distillate tested for skatol and indol by means of Ehrlich's aldehyde. By means of the color reaction obtained, it was possible to form a judgment as to the proportions of indol and skatol present in this fluid, a portion of the originally prepared solution being employed as a control in making this comparison. It was found that the tints obtained from the distillate from the gut were so nearly duplicated by
the tints obtained from the original solution that they could not be distinguished. Although there was this close correspondence in the colors obtained by the action of the aldehyde there was a less close correspondence between the colors obtained on shaking out with chloroform, the distillate from the fluid of the gut yielding a reddish rather than a purple tint, a result which may perhaps be regarded as pointing to the presence of slightly less skatol than indol. It was noticeable that the concentration of the indol and of the skatol in the fluid of the loop was less than one-quarter as great (judging by the intensity of the color reaction) as that of the original solution.

It is evident from the foregoing experiment that it gave no indication that indol is absorbed more readily than skatol—a result which harmonizes well with what is known of the close resemblance between indol and skatol in respect to solubility and chemical constitution. It thus appears in a high degree improbable that differences in the rate of absorption of indol and skatol from the intestine can account for the preponderance of skatol over indol that is sometimes noted in the contents of the human colon.

There is some evidence that skatol is in general a later product of putrefaction than indol. Nencki recommends long standing putrefactive mixtures containing muscle fiber and pancreatic gland, in order to obtain skatol in fair quantities. I have many times noticed in the course of putrefactive experiments in vitro that skatol appeared several days later than indol. It is not certain that this fact helps to explain the finding of skatol at lower levels of the intestinal contents than indol, because the conditions of decomposition within the intestine are so different from those that are experimentally induced. Experiments made on human subjects with the aid of cathartics indicate that proteid food may yield skatol within 24 hours, whereas, on artificial culture media, I have never observed it before the lapse of several days. Still it is likely that although both indol and skatol are sometimes more rapidly formed in the intestinal tract than in experiments in vitro, the relatively later formation of skatol is also a feature here. This seems the most reasonable explanation of the occurrence of skatol in the feces without indol, in those cases where indol has certainly been formed and absorbed.

1 Vortheilhafte Darstellung des Skatols, Centralbl. f. d. med. Wiss., No. 47, p. 849, 1878; Opera omnia, p. 433.
As yet I have not had a sufficiently long experience to make a
generalization with respect to the clinical conditions under which
the intestinal contents persistently show the presence of skatol in
excessive quantities. I have not found skatol abundant and
persistent in the feces except in the case of persons who are ill
or have recently been ill of some intestinal disorder. For this
reason I have come to attach to its presence more significance
than to the presence of indol, which is not infrequently found in
the intestinal contents of persons in apparently good health, who
are unconscious of any digestive disturbance. As to the kind of
proteid food most apt to favor the production of skatol no definite
statement can be made beyond the fact that a milk diet does not
necessarily cause skatol production to cease, although it seems to
render it less active than where the diet contains an equal quan-
tity of nitrogen in the form of meat proteid.

Where skatol formation is active in the intestine there are
usually other indications of excessive putrefaction, especially an
increase in the ethereal sulphates of the urine and an excessive
formation of hydrobilirubin within the gut. Indol production is
usually excessive but I have observed instances in which this
was not the case, instances in which the excessive putrefaction was
skatolic rather than indolic in type. This is not usually a transi-
tory phenomenon but is likely to be a long persistent feature.

The excessive formation of skatol I have found especially in
what I have described as instances of chronic excessive saccharo-
butyric intestinal putrefaction. Mental or emotional depression
has in some of these cases been the most persistent clinical feature;
in others there has been a moderate or considerable degree of
simple anæmia. In still others there have been present the blood
changes characteristic of pernicious anæmia. I have come to
believe that the chronic digestive disturbances of pernicious anæ-
mia are almost regularly associated with an excess of skatol in the
feces. In a case of appendicitis which came under my observa-
tion the feces contained skatol for several weeks after an operation
for removal of the appendix; with return to health this substance
gradually disappeared. In a patient with multiple neuritis
associated with persistent constipation and great production of
intestinal gases, skatol was regularly obtained in abundance from
the feces during the period of paralysis. With gradual convales-
cence there was a complete disappearance of the skatol of the
feces. Several other examples could be mentioned which illus-
trate the disappearance of skatol from the feces, concomitantly
with a betterment in clinical conditions. Apparently it would be
worth while to make careful systematic observations on the quan-
tities of skatol obtainable from intestinal material derived from
a variety of patients suffering from intestinal disease, with a view
to learning in how far the presence of this substance is a guide to
the intensity and course of bacterial processes in the intestinal
tract.

In practice I have found it convenient to make use of the following
mode of procedure in examining human feces for skatol. Twenty grams
of fresh material are ground in a mortar with a convenient quantity of
water. The suspension is then diluted up to 300 cc. It is now acidified
with phosphoric acid and distilled, the distillation being continued until
it no longer gives any color reaction with paradimethylamidobenzaldehyde.
A portion of the distillate is now treated with a paradimethylamidobenz-
aldehyde solution made up by dissolving 15 grams of the aldehyde in 30 cc.
of concentrated sulphuric acid and diluting this to 100 cc. The aldehyde
solution thus prepared is added to the distillate until the maximum color
reaction appears. This may be somewhat heightened by the addition of
a small amount of concentrated hydrochloric acid. If indol is present in
the distillate it should be removed by means of β-naphthaquinone-sodium-
monosulphonate.1 If phenol is present it must be got rid of by redistil-
lation, it being held back by strong alkali. For clinical purposes the quan-
tity of skatol present may be closely enough approximated by comparing
the color obtained with Ehrlich's aldehyde with various dilutions of a
watery solution of skatol of known strength. It is best to make the com-
parison after the contents of the test-tube have cooled, as this causes a
deepening of the color toward blue.

I have made experiments with many kinds of bacteria (using
pure cultures of bacteria and also using mixtures of bacteria) in
the hope of learning something of the conditions under which
skatol is formed rather than indol. Only a fair measure of suc-
cess has been gained in this attempt. The main obstacle to
success is the difficulty in obtaining a culture medium really com-
parable to that which the skatol-making bacteria find within the
human intestine. If we inoculate ordinary culture media (pep-
tone bouillon, plain agar, blood agar, milk) with mixed fecal

A Method for the Quantitative Determination of Indol, this Journal, i,
p. 257, 1906.
bacteria from a specimen containing skatol we are almost certainly disappointed in the hope that skatol will result in the course of the subsequent incubation. The putrefactive decomposition yields indol, not skatol. Only in rare instances is a trace of skatol found. From fluid media containing ground brain substance or fibrin one may obtain skatol, but the yields are seldom considerable and the putrefaction is usually indolic rather than skatolic. Sometimes after long putrefaction there is a considerable yield of skatol.

Difficulties have also been encountered in securing pure cultures of microorganisms capable of regularly giving rise to skatol when grown upon ordinary culture media. Marked irregularities in skatol formation have in some instances been observed where it has not been possible to accurately determine what conditions have been responsible for these irregularities. Nevertheless a few organisms out of a large number that were tried were found to produce skatol with considerable regularity when grown on peptone bouillon to which blood has been added. These organisms were a strain of malignant edema obtained from Prof. Theobald Smith, two strains of *B. putrificus* and an unidentified putrefactive anaerobic organism sent me by Dr. Smith. It is noteworthy that the best success in obtaining skatol has come from the use of organisms that grow under anaerobic conditions. Some strains of *B. proteus* of Hauser are probably also to be regarded as skatol-producers. There are doubtless many other anaerobic bacteria, besides those mentioned, which are capable of forming skatol. I have not been able to satisfy myself that *B. aerogenes capsulatus* ever produces skatol, though some strains make indol. As to tetanus, I am also in doubt. I have never been able to obtain more than mere traces of skatol from any strain of colon bacillus in my possession, whether the organism was grown aerobically or anaerobically. Long cultivation was required to give even these insignificant traces.

It is certain that the conditions which lead to the formation of indol are fundamentally different from those leading to the production of skatol. The conversion of skatol into indol is one that might be thought of as possible for microorganisms to effect. I have made experiments with a view to determining whether colon bacilli which were energetic indol-formers could form indol.
from skatol. The organisms were grown on a medium consisting of gelatin and salts to which a small quantity of skatol had been added. The organisms grew abundantly on this medium but even after many weeks' growth not a trace of indol could be detected. This result is in harmony with the observations made by Ellinger on indol and skatol formation in the intestinal tract of the rabbit. He states that at most a mere trace of indol may possibly be formed from skatol. I question whether even this is likely. Indol has been produced from skatol by potash fusion but so severe and destructive a method as this cannot be compared with any powers which it is likely that bacteria can exert.

That both indol and skatol are derived from tryptophan cannot be doubted. One may easily satisfy oneself of the ability of tryptophan to yield skatol and indol by experimenting with media made up by the addition of tryptophan to gelatin, pure cultures of different bacteria being employed. There is no reason to suppose that any other constituent of the proteid molecule than tryptophan is able to yield indol and skatol. We have thus to look to the chemical constitution of tryptophan for a clue to the solution of the problem why skatol is sometimes formed and at other times indol. I cannot pretend to offer an adequate hypothesis upon this question, but would like to call attention to certain facts which point to the reasonableness of the idea that the formation of skatol in one case and the formation of indol in another, may really be conditioned by the nature of the intermediate products that arise before tryptophan can yield either one of these substances. It may now regarded as settled that tryptophan is indol-amidopropionic acid and not skatol-amidoacetic acid, as was thought more likely by Hopkins and Cole. Moreover it is probable that tryptophan is an α-amido acid. It is certain that under the action of microorganisms tryptophan is capable of yielding indol-acetic acid, as was shown by Hopkins and Cole to be true of a tryptophan medium inoculated with a pure culture

of *B. coli communis*. This substance is probably not readily attacked by microorganisms. Some bacteria appear unable to attack indol-acetic acid. Thus *B. coli communis*, although able to form this substance from tryptophan either makes no skatol from it or only minute quantities. It is however probable that some bacteria (especially putrefactive anaerobes) are able to act upon indol-acetic acid in such a way as to cause it to lose carbon dioxide and such a change would explain the production of skatol. Assuming indol-acetic acid to be relatively unattackable by microorganisms, one would have an explanation of the usually small and slow yield of skatol in putrefaction.

Decomposition of tryptophan may, however, take another direction. Through oxidation, with the removal of the amido group, indol-propionic acid is formed. Assuming that this substance is relatively easily attacked by microorganisms, it is easy to see how indol and carbon dioxide might result from such bacterial attack, indol-carbonic acid being formed as an intermediate product. There is apparently no reason to suppose that indol-acetic acid is readily converted into indol-carbonic acid, since this calls for a process of energetic oxidation only likely to occur through the action of relatively powerful oxidizing agents. It would appear, then, that the formation of skatol may hinge on the antecedent production of indol-acetic acid, whereas the formation of indol may depend on the production of indol-propionic acid. To what extent this suggestion may be borne out by experimental facts can only be determined by further observations. Dr. Dakin has been so kind as to offer to prepare for me these important intermediary substances, the possession of which should serve to definitely determine their bacterial relation to indol and skatol.

The main conclusions which I desire to emphasize are the following:

1. Skatol is by no means always present in the contents of the lower gut in man. In healthy children it is only seldom detectable and then only in traces. In healthy adults it is frequently absent and when present occurs only in traces.

2. In some cases of excessive intestinal putrefaction skatol formation is considerably increased, often together with increased indol formation but sometimes without this.

3. There are instances in which the feces contain skatol but
no indol, despite the fact that the presence of indican in the urine points to indol formation in the intestine. As there is no evidence that indol is absorbed more rapidly than skatol in such cases, the presence of skatol without indol is probably due to the later production of the skatol.

4. Increased skatol production is observed in many persons suffering from excessive saccharo-butyric putrefaction due mainly to putrefactive anaerobic bacteria.

5. There are strains of the bacillus of malignant oedema and of *Bacillus putrificus* which form skatol. The *Bacillus coli communis* makes indol but usually no skatol or only mere traces.

6. The conditions giving rise to the formation of skatol are fundamentally different from those that govern the formation of indol. The formation of indol-acetic acid is perhaps a necessary step in the production of skatol, most bacteria attacking it with difficulty, if at all.
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