



THE DETERMINATION OF PHENOLS IN FECES AND FEEDS

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(Abstract)

During the past few years an investigation into the physiological action of phenolic contaminated drinking water has been carried on in this laboratory. Rats have been given phenol solutions in varying concentrations as a sole source of their drinking supply. The effects produced by these phenol waters upon growth, reproduction, food utilization, nitrogen balances and paths and characteristics of excretion have been recorded.

During the course of this investigation it became necessary to make phenolic determinations on the feces, feeds, urine and blood. The literature failed to disclose a method for determining phenols in the feces that would give results that could be tabulated, correlated, and compared with results showing the phenolic content of the urine obtained by F. F. Tisdall's ether extraction method (1). This latter method was therefore modified and used in the determination of phenols in feces, and with other slight variations, in feeds.

Any substance containing a benzene ring with a hydroxy group attached will react with the phenol reagent of Folin and Denis (2) which was used. Hence, results obtained include all phenolic substances. For this reason the word "phenols" become an inclusive term.

The above facts do not place this method at a disadvantage. The chief phenolic substances present in the excretions are tyrosine, phenol,

pyrocatechol, p-hydroxybenzoic acid, p-hydroxyphenyl acetic acid, and p-hydroxyphenyl propionic acid (1). The last five compounds are considered as decomposition products of the amino acid tyrosine. Thus, if tyrosine is removed before the final color is formed the results become a relative index as to the extent to which putrefaction is taking place in the intestines.

Since tyrosine gives the color it must be removed before making the final determinations.

To accomplish this, Tisdall devised his ether extraction method which was not done in the Folin and Denis method (3). The ether extraction has therefore been modified for the urine, feces, and feed. It should be noted that this method also removes tryptophane and uric acid, two substances which if present would cause the final results to be in error.

Procedure: Ten grams of feces, undried, are weighed into a 250 cc beaker. 75 cc of water are added and the mixture is allowed to stand for 45 minutes, with occasional stirring. It is then transferred quantitatively to a 200 cc volumetric flask. 20 cc of 10% sodium aluminum sulfate are added, followed by 1.5 cc of 5% lead acetate and the sample is diluted to the mark. It is allowed to stand for 15 to 20 minutes, with occasional shaking. The sample is then filtered and 10 cc aliquots are taken for analysis.

For free phenols, the aliquot is adjusted to pH, 6, and extracted directly with ether. For total phenols, 8 drops of concentrated hydrochloric acid are added to another aliquot and it is heated in boiling water for 10 minutes, the aliquot being placed in a test tube and covered with a small funnel. This heated sample must be cooled before the extraction is made.

Both free and total aliquots are now transferred to separatory funnels. Each is first extracted with 100 cc of ether, then extracted twice with 50 cc of ether each time. The phenols are extracted from the ether extraction with 25 cc of 10% sodium hydroxide. All extractions last for five minutes.

To release the color: The sodium hydroxide solution of phenols, (having been kept in the ice box between extraction and color determination) is made acid with concentrated hydrochloric acid. Two or three drops in excess are added. The aliquot is then transferred to a 100 cc volumetric flask and diluted to about 75 cc with boiling water. From 3 to 5 cc of phenol reagent are added, depending on the amount of phenol expected. 20% sodium carbonate is added until all acid is neutralized. This is shown when no more carbon dioxide is given off. 5 cc excess sodium carbonate are added and the sample is diluted to the mark. It is allowed to stand for one hour during which time the color forms and the sample cools. After cooling a final dilution is made and the color comparison is completed. A standard containing from .5 to 1 milligram of phenol in a total of 100 cc of solution proved satisfactory for the comparison. After the unknown sodium hydroxide solution has been neutralized the standard and unknown are given the same treatment.

The "phenolic" content of feeds is lower than that of the feces. Consequently, when feeds are analyzed, different dilutions and different strength standards are used.

It cannot be said definitely just what substance, or substances, present in the feeds will give this color reaction with the phenol reagent. Whether all or even any of this substance is phenol or of a phenolic nature is not known but certainly the increase of the total "phenols" over the free "phenols" cannot be properly called conjugated phenols.

It takes eight hours of boiling with 20% hydrochloric acid to break down protein. The acid concentration used is about 2% and the heating, in the case of feeds, is not more than 45 minutes in a water bath. Sub-

stances made available by this mild acid treatment will be made available by ordinary digestive processes. If any of this substance should be eaten by the rat and pass through its body unchanged, it should be found in our urine and feces determinations. The nature of the substance is still to be determined. It is present in a form that is water soluble before the treatment with acid, more soluble in ether than it is in water, its sodium salt is insoluble in ether and it produces color reactions with the phenol reagent.

REFERENCES

1. Tisdall, Frederick F. J. B. C., 44, 409-26 (1920).
2. Folin, Otto, and Denis, W., J. B. C., 12, 289-43 (1912).
3. Folin, Otto, and Denis, W., J. B. C., 26, 507-13 (1916).

