increase in alcohol and total acidity as compared with the uninoculated acidified medium.

No increase whatsoever of lactic acid was noted in any of the cultures. All of the organisms produced acids, but the quantity was small and variable. From 4.5 to 9.4 cc. *N* acid with an average of 7.0 cc. was produced by the non-pathogenic yeasts. The 12 cultures of monilia produced from 3.4 to 14.6 cc. *N* acid, with an average of 5.6 cc. The chief products of metabolism were CO_2 and ethyl alcohol. The average yield of alcohol from the 3 non-pathogenic yeasts was 94%. Excluding the results from organism 11, the monilia yielded 90% of alcohol. *Cryptococcus hominis* yielded 92% of alcohol.

Conclusions. The principal products of 13 pathogenic yeasts (12 monilia and *Cryptococcus hominis*) in a buffered peptone-meat-extract medium, with 5% glucose, were ethyl alcohol and CO_2. The yield of metabolic products was identical with that from 3 non-pathogenic yeasts.

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I. Quantitative Measurement of Coproporphyrin and Total Coproporphyrin I Excretion in Normals.

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Coproporphyrin I is excreted in the urine and feces under normal and most pathological circumstances.[1, 2, 3] In order to determine the relations between normal and abnormal pigment construction and destruction it has been necessary to develop an adequate method for the exact quantitative separation and measurement of porphyrins. Urine and feces were collected for one- to 3-day periods and kept in the dark. After careful mixing aliquot portions were analyzed. Not less than 50 gamma of coproporphyrin should be available for measurement; normally this amount is present in about half the daily amount of urine and about one-quarter of the daily feces. The

[1] Dobriner, K., *J. Biol. Chem.*, 1936, **113**, 1.

[2] Dobriner, K., *J. Biol. Chem.*, in press.

[3] Watson, C. T., *J. Clin. Invest.*, 1935, **14**, 110; 1936, **15**, 327.

remaining urine and feces were extracted for determination of melting points.

The reaction of the aliquot was made negative to Congo red by 10% sodium acetate. One-third of the volume of glacial acetic acid was added and the mixture shaken 3 times with 3 volumes of ether. The combined ether extracts were washed twice with dilute sodium acetate solution and the total porphyrin extracted quantitatively with 5% HCl. With sodium acetate and acetic acid the porphyrin was driven again into ether and extracted repeatedly and quantitatively from the ether after washing with dilute sodium acetate by equal volumes of 0.5% HCl. The 0.5% HCl was diluted to 0.2%, shaken repeatedly with alcohol-free chloroform until the chloroform was colorless. In the 0.2% solution only coproporphyrin is present. This is driven quantitatively into ether by means of sodium acetate and acetic acid. The ether was washed twice, extracted 3 times with small quantities of 5% HCl. These extracts were combined and brought to volume. A volume was chosen which gave a concentration of porphyrin about the same as the standard solution.

For the standard solution a 1.0 mg. % coproporphyrin I solution in 5% HCl was used. For the quantitative estimation a photoelectric colorimeter was employed (Goudsmit and Summerson[4]). Since traces of pigment are occasionally present which can be removed only with loss of porphyrin a combination filter from Corning Glass No. 349 and No. 429 was employed, allowing light 5320 to 6000 Ångstroms. Calculations may be made according to the usual methods of colorimetry. Check determinations are in agreement provided the extraction methods are uniform.

Quantitative determinations of urinary and fecal porphyrins were made in 5 normal adult patients over 9-day periods. The hematologic pictures and the amounts of urobilin excreted were within the normal ranges, indicating that the construction and destruction of respiratory pigments were in equilibrium. The daily excretions of coproporphyrin I varied moderately. The daily average excretions of coproporphyrin I are listed in Table I. The average of at least 6 consecutive daily determinations can be accepted as significant. The average values for the 5 adult cases studied fall within the same range, with a mean deviation of $\pm 10\%$, whereas in the case of a child the values were somewhat lower.

Type I porphyrins cannot be derived by degradation of any of the known respiratory pigments, which are all Type III compounds.

[4] Goudsmit, A., and Summerson, W. H., *J. Biol. Chem.*, 1935, **111**, 421.

TABLE I.
Quantitative Coproporphyrin I Excretion in Gammas in Average Daily Values for 9-day Period.

Case No.	Age	Sex	R.B.C. in millions	Hgb., gm. per 100 cc.	Coproporphyrin in gammas Urinary	Fecal	Total
1	12	F	4.5	15	35	191	226
4	21	M	4.6	13	87	231	318
2	25	M	4.5	15	123	205	328
5	33	M	4.59	14.4	102	274	376
3	35	M	5.08	15.9	121	241	362
6	72	M	4.3	14.8	64	242	306

They must be formed by synthesis of simple pyrrol complexes.[5] The simultaneous construction of Type I and Type III porphyrins, a phenomenon described in humans,[5] dogs,[6] and yeasts,[5] is called the dualism of the porphyrins.

Experimental observations as well as theoretical considerations suggest the following working hypothesis. *In vitro* porphyrins are formed by combination of substituted pyrrols to suitably constructed pyrromethenes. Two pyrromethenes are then condensed to porphyrins and mixtures of isomeric compounds are obtained. *In vivo* by enzymatic action or otherwise porphyrin synthesis is directed to the formation of large amounts of Type III porphyrins and a small amount of Type I porphyrin. Physiologically there is a direct proportion between the construction of Type III and Type I porphyrins. Simple increased or decreased Type III porphyrin construction associated with hematopoietic activity leads to a proportionally increased or decreased Type I porphyrin construction and excretion. Coproporphyrin-I excretion may, therefore, be an index of hematopoietic activity. In certain pathological states this fixed relation is disturbed so that a disproportionate Type-I construction occurs.[7] Finally, faulty construction or utilization of Type III compounds may lead to the production of Type III porphyrins.

[5] Fischer, H., *Verhandl. deutsch. Ges. inn. Med.*, 1935, **45**, 7; *Handbuch der normalen u. pathologischen Physiologie*, Berlin, 1928, **6**, 164; 1932, **18**, 148.

[6] Dobriner, K., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 757.

[7] Dobriner, K., Localio, S. A., and Strain, W. H., *J. Biol. Chem.*, 1936, **114**, XXVI.