REPORT

of

Committee on Thesis

The undersigned, acting as a Committee of the Graduate School, have read the accompanying thesis submitted by Charles Ulysses Moore for the degree of Master of Science They approve it as a thesis meeting the requirements of the Graduate School of the University of Minnesota, and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science

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PHENOL EXCRETION IN INFANTS INCLUDING NEWBORNS.

A Thesis Submitted to the Faculty of the Graduate

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by

Charles U. Moore.

In partial fulfillment of the requirements for

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PHENOL EXCRETION IN INFANTS INCLUDING NEWBORNS.

Our knowledge of phenol begins with its discovery in 1834 by Runge.[1] He obtained it while distilling coal tar and therefore called it "carbon-oil acid" or carbolic acid. Also he observed the physiological properties that it possesses in common with creosote. Laurent[1] was the first to obtain it pure and gave it the name hydrate de phényle, or acid phenique from phainein - to illuminate, probably because it occurs in the tar produced in the manufacture of illuminating gas. Gerhardt,[1] who prepared it from salicylic acid, introduced the name phenol, indicating thereby that it is an alcohol.[1] The number of phenols that have since been isolated amounts to many hundreds (Bielstein)[2].

Rosenthaler in "Der Nachweis Organischer Verbindungen" (1914) gives the following as the most important of the phenols:

Monohydric phenols

Phenol	C_6H_5OH
(o.m.p.) Cresol	$C_6H_4CH_3OH$
2-Aethylphenol (phlorol)	$C_6H_4C_2H_5OH$
Xylenol	$C_6H_3(CH_3)_2OH$
Carvacral	$C_6H_3CH_3.C_3H_7OH$
Thymol	$C_6H_3CH_3.C_3H_7OH$
Chavical	$C_6H_4C_3H_5OH$
A- & B- Naphthol	$C_{10}H_7OH$

Dihydric phenols

Pyrocatechin $C_6H_4(OH(1))(OH(2))$

Resorcin $C_6H_4(OH(1))(OH(3))$

Hydrochinon $C_6H_4(OH(1))(OH(4))$

Trihydric phenols

Pyrogallol $C_6H_3(OH(1))(OH(2))(OH(3))$

Phloroglucin $C_6H_3(OH(1))(OH(3))(OH(5)) + 2H_2O$

Oxyhydrochinon $C_6H_3(OH(1))(OH(2))(OH(4))$

The most important phenol in the urine is para-kresol (Siegfreid and Zimmerman, Bauman, and Mooser)[3]. In addition there is some orthocresol, pyrocatechin, hydroquinone, para-oxybenzoic acid, and probably others.

Lister[4], of Glasgow, in 1867 showed the great importance of phenol as a disinfectant. Its use at this time in strong solutions, both as a surgical dressing and as a spray in the operating room, was followed in many cases by dark urines and symptoms of poisoning. "Carbolic acid gangrene" was not uncommon.

The chemists of this period became deeply interested in the origin and the fate of phenol in the animal organism. G. v. Städeler[4], in 1851, working with cattle urine, was the first to discover that phenol is excreted by normal animals.

In considering the origin of phenols in the animal organism, one must turn to the accurate observations of Bauman[5]. In

determining the sulphates of the urine he used a measured quantity of urine, strongly acidified with acetic acid, to which he added $BaCl_2$, heated it on the water bath for an hour and then filtered. He then added to the filtrate one-eighth of its volume of concentrated HCl and again heated on a water bath. This gave a second precipitation of $BaSO_4$ which he rightly reasoned, came from organic sulphates.

Bauman proved that the organic substances which unite to form the conjugated sulphates are phenol, pyrocatechin, and some other aromatic substance, later found to be glucuronic acid. To see whether phenol and sulphates given separately would unite within the organism, he gave Na_2SO_4 with the food and applied phenol externally. The result was a twenty-fold increase in the output of phenol-sulphuric ester.

To ascertain next where in the body phenol sulphates are formed, he examined the blood one-half hour after the intravenous injection of phenols (no Na_2SO_4 having been given) and found large amounts of free phenol with only small amounts of "phenol forming substances" or conjugated phenols. Two hours later the proportion was interchanged. Also 100 gm. of liver then contained 19 times as much conjugated phenol as did a like amount of blood. However, the phenol sulphuric acid disappeared from the liver quite rapidly. An examination of the liver of normal horses gave no phenol and four liters of normal horse blood gave only very small traces. The kidney likewise gave negative results.

In the urine of herbivorous animals one finds much larger amounts of phenol than is the case with purely carnivorous animals. In one liter of horse urine Munk[6] obtained .913 gm. of phenol;

Mooser[3] obtained .25 - .77 gm. of para-kresol but no phenol. In contrast to these urines of herbivorous animals, Jonescu[7] found no phenol in the urine of a dog which had been fed on meat alone.

This fact that urine of herbivorous animals gave larger amounts of phenol suggested a vegetable origin but Bauman's results in dogs and arterwards in man indicated that proteins were the source, tyrosin being considered the mother substance. Through the further work of Bauman and his pupils (Brieger[8] and Weyl[9]) it was found that phenol and indol were formed by putrefaction of the protein materials, that phenol was found in the large intestine, and that the increased amounts in the horse and cow were probably due to their longer digestive tract and consequent greater action of bacteria.

Tyrosine acted upon by putrefactive bacteria, under anerobic conditions, gave large amounts of phenol. Feeding tyrosine greatly increased the urinary phenols (Weyl[9]). Clinical observation also showed an increase of phenols in Ileus and other obstructive conditions.

The phenol output is largely increased, according to Salkowski[10] and Brieger[8], in stoppage of the bowel, especially of the lower part of the small intestine and in the large intestine, in peritonitis, in pyemia, in abscesses, and other purulent conditions, and, according to Blanderman[11], in phosphorus poisoning. Strasser[12] obtained, by the method of Kossler and Penny in anemia, phosphorus poisoning, icterus, cystitis, and cirrhosis of the liver 36-50 mg. of phenol as the daily excretion. In putrid bronchitis he obtained 51 mg., in diabetis without acetone 91, in face erysipelas 96, in nitro-benzol poisoning 97. Pneumonia averaged in four determinations 110 (68-188), typhus, during the fever 142

and, after a subsidence of the temperature, 35-39 mg.; pyopneumothorax gave 194 mg., and gangrene of the feet 261 mg.

Russo[13] found in his investigations of the urines of thirty patients a daily maximum of 225 mg. In cirrhosis of the liver without icterus he found no phenol, in tuberculosis of the lung only traces, and in diseases of the bowel without stoppage no increase. Herter and Wakeman[14] obtained as much as 790 mg. in diabetis (possibly due to carbohydrates).

In contrast to these high results obtained in diseases, the daily amount of phenol excreted in normal human urine, according to Munk[6], varies from 17-51 mg. An average of the findings of Munk, Neuberg, and Mooser amounts to 30 mg. daily[15]; while Kossler and Penny[16] obtained as much as 70-106 mg. in persons on a mixed diet. In experiments made on nine healthy students Siegfreid and Zimmerman[17] obtained 44.6 mg. as the daily average.

Of the many methods advanced for the estimation of phenol in the urine the first to lay claim to accuracy was that devised by Messinger and Vortman, with a modification by Kossler and Penny[16], and was conducted in the following manner:

500 c.c. of urine, made weakly alkaline, is evaporated over a water bath to one-fifth its volume, during which process the acetone escapes. The remainder is again restored to its original volume by the addition of water; then to each 100 c.c. of the liquid one adds 5 c.c. of concentrated H_2SO_4 and distills to one-half the volume. The amount thus lost is replaced by water and the distillation is repeated several times, in this manner. "The vessel may be open during the distillation without the loss of phenol." The formic and nitric acids are removed by shaking the

distillate with K_2CO_3 until the acid reaction disappears. The solution is distilled off from the K_2CO_3 and the salt remaining after the distillation of the first portion is used for neutralizing the succeeding portions. The distillates are made alkaline with a measured amount of deci-normal alkali; are heated by immersion in water at 60°, and are then treated at once with deci-normal Iodine solution, the amount added being 10-15 c.c. more than the amount of alkali used. After cooling, the sublimated Iodine is rinsed off the wall of the flask with the solution, is acidified, and the excess of iodine is titrated back with thiosulphate solution.

Modification of Neuberg.[18]

Neuberg ascertained that the phenol values obtained according to Kossler and Penny are far too high in the presence of carbohydrates or other substances which on heating with acids give volatile Iodine-binding substances, because these are also estimated as phenols. He therefore recommended the following modification.

The solution containing phenols and the other components of the carbohydrates that unite with iodine, is purified over $CaCO_3$ and is treated with a solution of one gm. caustic soda and six gm. of sugar of lead. The flask is then connected with a distilling apparatus and heated fifteen minutes over a vigorously boiling water bath. For a complete removal of the aldehydes, one heats the contents of the flask a short while longer over a free flame, until a few c.c. of the distillate no longer reduce a silver solution made alkaline with NH_4OH. Thereupon the solution is strongly acidified with H_2SO_4 and again distilled. The distillate is made alkaline and treated further according to Kossler and Penny.

In dog urine containing sugar Neubauer and Huppert[19] found that this method occasionally failed since one can not distil off all the aldehyde-like substances that reduce silver solutions without the loss of some of the phenol.

In cow urine Leichti and Mooser[20] have ascertained losses of more than 50 per cent of the kresol present when they distilled off all the furfurol. Hensel[21] noted similar losses in sugar-containing human urine according to the Neuberg method.

Modification of Mooser.

Mooser came to the conclusion by testing the method of Kassler and Penny with pure phenol and kresol solutions that a loss of both substances results. He further found that in the distillation of pure kresol solutions with H_2SO_4 that not all of the kresol is re-obtained from the distillate. He distilled therefore with H_3PO_4 and took up the second distillation with $CaCO_3$ in a carbon dioxide stream. In this manner, upon titration of the distillate, exact values were obtained.

Mooser's method[22] is as follows: A measured quantity (250-500 c.c.) of urine, made weakly alkaline, is evaporated over a water bath to about one-fifth of its volume, is rinsed into the distillation flask, and this is connected with a distilling apparatus. Through a special cock-funnel, which at the same time serves as part of the distilling apparatus, one slowly introduces, with occasional shaking, enough of the syrupy H_3PO_4 to make the volume equal to about one-fifth of the original volume. After thorough cooling, it is distilled to about 100 c.c. and the distillation, with occasional additions of 50 c.c. of water, is con-

tinued until a test of a few drops of the distillate with Millon's reagent results negatively. The distillates, caught in a large flask, are subjected to a renewed distillation after supersaturation with K_2CO_3 by the introduction of a stream of pure CO_2. This is repeated until the distillate no longer gives a Millon's reaction. The distillate is titrated according to Kossler and Penny. "It is to be noted that owing to the carbonic acid contained in the distillate, the quantity of NaOH added must be increased accordingly."

Method of Ellinger and Hensel.[20]

The distillate obtained according to Mooser's method is treated with H_3PO_4 and thoroughly shaken up with ether. The ether solution is then freed from volatile acids and shaken with dilute NaOH until the phenols have completely gone over into the alkali. In the alkaline solution, after neutralizing most of the free alkali with H_2SO_4, the phenols are titrated according to Kassler and Penny.

Colorimetric Methods.

The following three colorimetric methods have been found of value where pure phenol (C_6H_5OH) only is desired.

Bonanni[23] determined the "extinktion koefficient" and the relation of the absorption of the colored combinations which phenols form with iron chloride, and recommended these constants as a direct means of determining the phenol in the urine.

Bordas and Robin[23] conducted the phenol from the urine distillate, either by warming with saturated KNO_3 solution and concentrated H_2SO_4, over into Picric acid and determined these colorimetrically as ammonium picrate; or used, for a colorimetric determination, the red color that arises when watery phenol solu-

tions and nitrogen dioxide are heated together with saturated H_2SO_4.

Kiesel[23] distills the urine with H_2SO_4 and supersaturates the distillate with soda, after which he distills again. In the second distillate the intensity of Millon's reaction is compared colorimetrically with a normal solution which contains three parts kresol and one part phenol.

Folin and Denis[24] have recently shown that all methods by which alkaline urine is condensed in volume cause a part of the phenol to be oxidized and thereby render abnormally low results. They have developed a new method by which no phenol is lost by evaporation or oxidation and the results are, consequently, much more accurate.

Their method is as follows: "Ten c.c. of concentrated or 20 c.c. of very dilute, urine are placed in a 50 c.c. volumetric flask. To this is added an acid silver lactate solution (3 per cent silver lactate solution in 3 per cent lactic acid) until no more precipitate is formed. A few drops of colloidal iron are then added, the flask shaken, filled to the mark with distilled water, shaken again, and its contents are filtered. 25 c.c. of the filtrate are transferred to a 50 c.c. volumetric flask, and to it is added a sufficient quantity of saturated sodium chloride solution, containing 10 c.c. of strong hydrochloric acid per liter, to precipitate all the silver. The flask is then filled to the mark and the contents are filtered.

"To determine 'free' phenols 20 c.c. of this filtrate are placed in a 50 c.c. flask and treated with 5 c.c. of our phosphotungstic, phosphomolybdic acid reagent[25] and 15 c.c. of the satu-

rated sodium carbonate solution. After diluting to volume with lukewarm water (30° - 35° C.) and allowing to stand for twenty minutes the deep blue solution is read in a Duboscq colorimeter against a standard solution of phenol.

"To determine total (free and conjugated) phenols 20 c.c. of the same filtrate are transferred to a large test-tube; to this are added ten drops of concentrated hydrochloric acid, and the test-tube is covered with a small funnel. This mixture is heated rapidly to boiling over a free flame and is then placed in a boiling water bath for 10 minutes. At the end of this time the tube is removed, cooled, and the contents are transferred to a 100 c.c. volumetric flask. 10 c.c. of our reagent and 25 c.c. of saturated sodium carbonate are now added, and after making up to volume and shaking, the solution is read (after twenty minutes) against a standard solution of pure phenol. As a standard we use a solution of pure phenol in N/100 HCl containing 1 mg. of the former substance in 10 c.c. 5 c.c. of this solution (equivalent to 0.5 mg. of phenol) when 10 c.c. of saturated sodium carbonate solution are added and the whole is made up with water at about 30° C. to a volume of 100 c.c., give, when set in the colorimeter at 20 mm., a convenient standard."

The results obtained by the Folin and Denis method in adults were so much higher than those obtained by former methods that I became interested in learning what the results in infants would be. Encouraged by Dr. Folin I began the study of phenol excretion in infants. Later, at the instigation of J. P. Sedgwick, I undertook to prove or disprove the statement made by Senator[26] and others, that phenol is not present in quantitative amounts in the urines of newborns.

The urines of infants are usually of low specific gravity, while those of newborns are very concentrated. For this reason I found it necessary to vary the amount of urine used for a test. In infants 20-25 c.c. were usually required, while in newborns 3-5 c.c. gave the best results. Large amounts of a concentrated urine cause an unfilterable colloidal silver precipitate not found when proper amounts are used. Also one's readings are thereby easily within the limits of the colorimeter.

A change was made in Folin and Denis' method so as to make the results more uniform in both cold and warm weather, and also to get the maximum blue color in the shortest possible time. After adding the reagent and the Na_2CO_3 solution to the 50 c.c. flask, one is directed to dilute to volume with lukewarm water. As the amount of lukewarm water to be added to the flasks varies, they are heated to different degrees and this makes a marked difference in the time of the appearance of the maximum blue color. I, therefore, filled all flasks with water at room temperature and then placed them in a water bath with a temperature of 40°-45° C. Thus all are warmed equally and in ten to fifteen minutes the maximum color was reached.

With infant urines the method is carried out as follows: 20 c.c. of urine are placed in a 50 c.c. volumetric flask and usually 15 c.c. of the silver lactate solution added plus a few drops of colloidal iron, the flask shaken, filled to the mark, inverted four or five times, and then the solution filtered. Often a little of the precipitate goes through the filter paper at first. If so, this is poured through the filter paper again. 40 c.c. of the filtrate are placed in a 50 c.c. flask and 5 c.c. of the acid

sodium chloride solution added, filled to the mark, shaken, and filtered.

20 c.c. of this second filtrate are then placed in a 50 c.c. flask to be used for the determination of free phenols. With the same pipette and with solutions at the same temperature, 20 c.c. more are put into a 200 x 25 m.m. test-tube for the total phenol determination. A funnel is then inserted in the test-tube.

Instead of heating the test-tubes over a free flame I place them directly into a boiling water bath (600 c.c. beaker) and let them boil for one minute. I then use a microburner under the beaker to keep the water hot, but not boiling, for fifteen minutes.

The test-tubes are then cooled and rinsed into a 50 c.c. volumetric flask, and 6 c.c. of the reagent and 15 c.c. of a saturated solution of Na_2CO_3 added. The free, total, and standard solutions are all treated with the reagent and with Na_2CO_3, at the same time filled to volume, and then placed in a basin of water at 40° to 45° C. After remaining here until an intense blue color develops (usually 15 minutes), all are then centrifuged in a high-power centrifuge for two minutes to make the solutions absolutely clear. Readings are then made in the colorimeter in the usual way.

The large test-tube into which the funnel is placed performs a double function - that of a digesting and of a condensing apparatus. It should therefore receive some attention to avoid the loss of phenols by evaporation. The water bath is prepared by using a tall 600 c.c. beaker without a lip. Into this beaker is placed a wire gauze so as to support the test-tubes about an

inch from the bottom. This allows the top of the test-tubes to extend over the sides of the beaker, by means of which the top remains cool enough to act as a good condenser. Enough water is placed in the beaker to reach nearly the level of the liquid in the test-tubes.

This gives absolutely reliable results as I have proved by testing with known phenol solutions and also by the use of a reflux condenser. By this technic I found that my total phenols were always greater than the free phenols. Whenever the free and total were equal, carefully repeated tests proved this to be due to a technical error.

I have carried out experiments on fifteen infants making daily examinations, in several cases, for a period of three weeks. In none of these during the time of investigation was there any hyperpyrexia so I have omitted temperature records from the following tables.

In Table 1 I have included all factors so far as I was able to ascertain them which would affect the infants' metabolism, and especially those which might influence their excretion of phenols. I have to thank Dr. Fitz B. Talbot of Massachusetts General Hospital, Dr. Henry I. Bowditch of the Boston Floating Hospital, and Dr. J. P. Sedgwick of the University of Minnesota Hospital, for urine specimens and data on these cases.

The diets in numbers 4-12 inclusive were prepared in the Walker-Gordon laboratory in Boston. In those cases where homogenized milk was given the proportions of the food elements were estimated with great accuracy.

Homogenizing was accomplished by means of the agate valve

TABLE #1.

INFANTS.

No. Name. Sex.	Age. Clinical Condition.	Weight with gain or loss for week	DIET. Amount Kind. % Fat C'hydrate. Protein.	Stools No. Form.	URINE. Volume cc	Specific Gravity	Total Nitrogen gm.	PHENOLS. Free mg.	Total mg.	Free %	Conjugated %
#4. Lamachia ♂	3 mos. Under-nourished.	4300	1050 cc. Modified Milk. 2.5-6-1.25		780	—	2.36	54.2	61.5	88	12
					630	—	3.42	43.1	54.6	79	21
			Average for week-		705		2.89	48.6	58	83	17
#5. Casey ♂	6 mos. Healthy.	3550	1050 cc. Modified Milk. 1-5.6-1.5		750	—	1.16	40.4	42.6	94	06
					600	—	1.01	39.6	43.3	91	09
					930	—	1.62	72.7	80.6	90	10
			Average for week-		726		1.26	50.9	55.8	91	09
#6. Walsh. ♂	6 mos Healthy	5340	1050 cc. Modified Milk 2-6-1.75	3, L	855	—		29.2	35.3	80	20
				1, L	780	—		25.7	31.2	80	20
				1, L	840	—		34.8	36.2	96	04
				3, 1	720	—		25.6	30.6	83	17
				3, 1	780	—		34.4	40.9	84	16
				2, L	740	—		37.1	39.6	93	07
		-60		1, L	825	—		28.4	39.3	72	28
		Average for week-		2	790		2.91	30.6	35.6	86	14
		5260	1050 cc Modified Milk Homogenized 2-6-1.75	3, —	780	1008	1.394	21.6	26.2	83	17
				4, L	810	1010	1.110	23.4	27.4	86	14
				2, L	750	1009	1.471	30.6	33.3	92	08
				3, L	810	1007	2.002	27.6	32.9	80	20
					810	1008	1.929	31.6	34.9	90	10
				5, L	660	1011	1.788	21.4	30	70	30
		no change		3, L	705	1009	1.659	28.6	32.3	88	12
				2, L	780	1005	1.954	24.8	32.5	66	34
		Average for week		3	763	1007	1.650	26.2	31.2	84	16

R.T.

II. TABLE #1.

INFANTS.

#6. Walsh (continued) ♂	6 mos. Healthy.	5260	1050 cc. Special Homogenized 2-6-1.75	2, L	870	1011	2.179	30.2	39.9	76	24
				2, L	750	1011	1.488	27.9	32.3	86	14
				1, L	840	1012	1.835	17.9	22	81	19
				1, —	810	1008	1.053	17.	25.3	67	33
			(the 2% fat is dive oil)	1, L	840	1006	2.151	19.5	29.	67	33
				1, L	840	1012	2.141	19.7	29.6	67	33
				1, L	780	1012	1.945	15.4	24.7	62	38
		-180	Average for week -	1.3	790	1010	1.912	20.7	29.1	65	35
			Average of 22 determinations -	2	781		1.917	25.8	30.9	83	17
#7 Keddish. ♀	2 mos Normal.	3095	630 cc. Breast Milk 4-7-1.5	, L	480	1004	.318	10.	13.2	75	25
				, L	480	1004	.586	9.6	11.2	85	15
				8, —	465	1005	.895	15	17.6	85	15
				5, —	465	1006	.650	8.1	15.3	53	47
				4, —	465	—	.706	9.3	11.	84	16
				6, —	420	—	.513	8.4	12	70	30
		+270		5, —	480	1010	.524	9.2	11.4	80	20
				6, —	450	1010	.575	9	11.8	76	24
				2, —	420	—	.501	10	11.8	85	15
			Average for week -	5.1	458	1006	585	9.8	12.8	76	23
		3265	630 cc. Breast Milk Homogenized 4-7-1.5	8, L	390	1008	.459	10.3	12.5	80	20
				4, —	420	1008	.513	15.2	17.0	89	11
				2, —	330	—	.400	7.9	12.1	65	35
				5, —	360	—	.437	8.3	10.4	80	20
		+90		3, —	420	—	.464	9.6	13.9	79	21
				3, —	390	—	.387	8.1	11.	74	26
			Average for week -	4.2	385	1008	.285	10.1	12.8	79	21

R.T.

-III- TABLE #1.

INFANTS.

#7. Reddish (continued) ♀	2 mos. Normal	3350	630 cc. Breast Milk 4-7-1.5	5, —	420	1008	.518	12.2	15.7	78	22
				6, L	300	—	.195	7.6	10.1	75	25
				3, L	360	1006	.464	8.8	12.3	78	22
				2, —	390	1006	.453	7.5	9.9	75	25
		+30	Average for week-	4	417	1007	.407	9	12	75	25
	Average of	19	determinations-	5+	420		.426	9.7	12.6	77	23
#8 Zucalla ♂	4 mos. Balance Disturbance	3265	945 cc. Special Homogenized (Olive oil 1.6%) 1.6-6-1.28	2, —	780	1010	1.355	19.	23.9	80	20
				6, —	840	1008	1.083	24.3	32.8	74	26
		-90		4, L	840	1010	1.072	22.2	31.9	70	30
			Average for week-	4	820	1009	1.170	21.8	29.5	74	26
		3890	945 cc. Modified Milk 1.6-6-1.28	3, 1	810	1010	1.593	35.8	47.2	78	22
				1, L	600	1012	1.239	26.6	36.1	73	27
		-120		2, 1	720	1008	1.481	35.2	42.2	83	17
			Average for week-	2	710	1007	1.437	32.5	41.8	78	22
		3835 (8%)	945 cc. Modified Milk Homogenized 1.6-6-1.28	6, —	630	1012	1.471	21.9	23.9	90	10
				9, —	390	—	—	17.4	20	87	13
				6, —	570	1010	1.257	17.	24.2	70	30
		+30		4, L	600	1010	1.244	12.1	17.4	69	31
				2, L	630	1011	1.339	13.3	25.9	55	45
			Average for week-	5½	560	1010	1.303	16.3	22.3	77	23
	Average of	11	determinations-	4	698	1009	1.303	23.5	31.2	76	23
#9 Adams ♀	4 mos. Normal	4290	960 cc. Special Homogenized (Olive oil 2% Barley Starch 3%) 2-6-1.6	4, —	750	1010	1.414	32.3	40.4	80	20
				2, L	720	1012	1.415	32.2	37.2	86	14
		+60		4, L	750	1010	1.618	34.2	39.8	86	14
			Average for week-	3.3	740	1010	1.482	32.9	39.1	84	16

R.T.

IV. TABLE #1. INFANTS.

#9. Adams (continued) ♀	4 mos Normal.	4430	960 cc. Modified Milk (Barley, Starch .75%) 2-6-1.6	7, L	510	1009	1.484	22.9	26.6	86	14
				8, —	600	1010	1.999	28.2	31.3	90	10
		+120		4, —	375	1012	1.187	18.7	23.5	79	21
		Average for week —		6.3	495	1010	1.557	23.3	27.1	86	14
		4430	960 cc. Modified Milk (Barley, Starch .75%) 2-6-1.6	6, —	570	—	1.482	28.5	31	92	08
				4, —	510	—	1.532	17.5	20.4	86	14
				1, —	570	—	1.480	15.5	18.7	83	17
				4, L	615	1010	1.547	16	25.6	62	38
		+60		3, L	630	1012	1.663	26.3	29.2	83	17
		Average for week —		3.6	579	1011	1.541	20.7	24.9	84	16
Average of 11 determinations —				4.3	605	1010	1.526	25.6	30.3	85	15
#10. Caples. ♂	6 mos. Acute Intestinal Indigestion.	5340	1080 cc. Modified Milk (Barley, Starch .75%) 2-6-1.75	6, —	780	1010	1.171	16.6	25.1	66	34
				8, L	840	—	1.864	18.4	26.6	69	31
		no change		9, —	720	1010	1.501	14.3	20	72	28
				5, —	855	—	1.557	21.1	24.3	87	13
		Average for week —		7	799	1010	1.523	17.6	24	73	27
#11. Gilman ♂	5 wks. Malnutrition	1765	750 cc. Breast Milk 8 parts Whey 2 " Lactose 6% 3.2-6-1.4	8, —	420	1004	.198	7.	9.	67	33
				5, —	450	1005	.260	8.7	11.4	76	24
		+90		10, —	510	1005	.279	8.9	10.6	84	16
				4, —	420	1008	.391	10.8	11.2	96	04
		Average for week —		7	465	1006	.382	8.8	10.5	83	17
#12. Crowley ♂	2 mos. "Colic" Well nourished	4680	735 cc Modified Milk (Barley Starch .75%) 2.4-6-1.9	2, L	450	1010	1.236	19.5	24.2	80	20
				3, L	690	1012	2.019	22.5	30	75	25
				3, L	660	1010	1.926	13.8	20.1	68	32
		+90		4, L	690	1014	2.618	13.5	16.4	80	20
		Average for week —		3	622	1011	1.975	17.3	22.7	77	23

V. TABLE #1. INFANTS.

#12. Crowley (Continued) ♂	2 mos "Colic" Well nourished	4770	735 cc. Special Homogenized (Olive oil 2.4% Barley starch 3.0%) 2.4-6-1.9	4, L	525	—	1.162	14.9	17.3	85	15
				4, L	630	1012	1.741	18.8	19.2	97	03
				4, L	600	1010	1.578	—	25.9	—	—
				3, L	480	1012	1.334	13.3	20	66	34
		-60		4, —	480	1014	1.465	14.3	23	67	33
		Average for week -		4	543	1012	1.465	15.3	21.1	73	27
Average of 9 determinations				3.5	583	1011	1.686	16.3	21.9	75	25
#13. Glover ♂	2 wks. Normal	2860	Breast Fed. 5 feedings (Amount not recorded)	1, L	120	1006	.265	8.6	11.1	77	23
		+40		1, L	180	—	.264	15.6	16.1	97	03
		Average for week -			150	1006	.264	12.1	13.6	89	11
#14. Vincent ♂ (# 72 hour specimen)	1 week Normal	2860	Breast Fed 5 feedings. (Amount not recorded)	2, L							
		+80		1, L							
				1, L	100	1022	.880	22.6	27.1	83	17
		Average for week -			34.		.293	7.5	9.	83	17
#18. Tucker. ♂	3 mos. Normal Under-nourished	3600	750 cc. Protien Milk +1% Dextri-Maltose	1, □	250	1012	1.100	14.9	17.3	87	13
		+120		2, L	200	1008	1.210	15.3	16.7	92	8
		Average for week -			225	1010	1.185	15.1	17	89	11
#20. Tollefson ♂	6 mos Cleft palate Otitis Media.	4950	750 cc. ½ Milk - ½ Oatmeal Water - +4% Sugar Cereal B.i.d.	1, L	240	1010	.947	24	27.5	87	13
				1, L	180	1014	1.436	37.7	40.1	94	6
		-50			210	1012	1.192	30.8	33.8	91	9
		Average for week -									

VI. TABLE #1.

INFANTS.

#21. Samuelson	6 wks. Healthy.	5740 +210	960 gm. Breast Milk	2, L	425	1010	.509	28.7	38.3	75	25
				2, L	380	1006	.316	28.4	35.2	81	19
			Average for week -		402		.412	28.5	36.7	78	22
#22. Voulus.	9 wks. Healthy	2840 -70	405 gm. Breast Milk	2, 1	155	1007	.358	18.6	21.6	87	13
			General Average		463		1.107	24	28.9	83	17

Characters indicating consistance of stools are as follows: 1, formed; L, intermediate or soft; —, watery; □, soap stool.

French machine at a pressure of 3000 pounds and was so thoroughly done that the cream would not rise on milk thus treated even after standing for weeks.

The results from homogenizing of whole milk or of olive oil mixed with fat-free milk show no effect upon the phenol excretion. This was to be expected since it has long been established that phenols arise from proteins.

The ages of these infants varied from one week to six months, their weights varied from 1765 grams to 5740 grams, the food intake from 405 to 1080grams, and the number of stools per day from one to nine.

The total phenol excretion per day ranged from 9 mg. in case No. 14 to 58 mg. in case No. 4. The average phenol excretion per day was 28.9 mg., of which 83 per cent was free phenol.

The consistence of the stools in my cases was apparently not a determining factor in the amount of phenols excreted. This agrees with the observations of Meyer[27] and with the statement of Freund[28] with reference to the output of ethereal sulphates.

H. Senator[24] in 1879 was the first to obtain phenol qualitatively in the urine of infants and he found only fleeting (*flucht-ige*) traces.

Ludwig F. Meyer[27] in 1905, using the method of Kossler and Penny, investigated the phenol excretion of seven infants, two breast-fed and five artificially fed. For the sake of ready comparison I have tabulated his data and present it in Table 2.

Table 2. L. F. Meyer's Cases.

No.	Age Weight, grams	Clinical condition	Diet	Stools	Phenol mg.
1	6 mos. 6330	Healthy	850 gm. Breast milk		5.87
2	10 wks.	Healthy	600 gm. Breast milk		2.507
			Average in breast-fed		4.19
3	7 wks. 3450	Healthy	Condensed milk "Schleim" & sugar	3 yellow	8.46
4	5-3/4 mo. 4350	Healthy	Solution of powdered milk	3 yellow thin	12.22
5	13 mos. 5740	Very rachitic	600 gm. 1/2 milk, 1/2 flour and gruel soup	3 yellow thin	13.16
6	4-1/2 mo. 4000		1/2 flour soup 1/2 powdered milk solution	4 yellow thin	15.67
7	10 mos. 5020	Underdeveloped, rachitic, perspiring greatly	Breast milk & buttermilk	1 yellow homogenous	16.92
			Average in artificially fed		13.28

In these cases the phenol excretion varies from 2.5 - 16.9 mg., the average for his breast-fed cases being 4.19 mg. and for the artificially fed 13.28 mg. He does not note the daily output of urine nor the total nitrogen. These figures of Meyer constitute the only quantitative investigations made on infants previous to 1915 that I have found in a thorough review of the literature.

Folin and Denis[29], in reporting results by their recent method, mention seven infants whose phenol excretion averages 48 mg. as shown in Table 3.

Table 3. Phenol Excretion in Hospital Infants.

(Folin and Denis)

No.	Age mos.	Clinical condition	Urine					
			Volume cc.	Total N. gm.	Phenols			
					Free mg.	Total mg.	Free %	Conjugated %
61	10	Malnutrition	860	1.3	42	43	98	2
63	4	Malnutrition	740	1.8	40	40	100	0
64	2	Malnutrition	550	1.6	68	68	100	0
65	2	Malnutrition	610	1.7	31	35	89	11
71	6	Malnutrition	755	1.5	40	42	95	5
60	3	Normal	600	1.3	30	50	70	30
66	10	Rachitic	620	2.9	50	60	77	23
	5.3		695	1.74	43	48	89	11

The weights of these infants are not mentioned nor is the number or character of their stools. The amount and character of diet are also omitted. However, the comparatively large excretion of urine, total nitrogen, and phenols would indicate, as compared with my own results, that these infants were given artificial food.

A rather striking fact is found in cases 63 and 64 in that the free phenols equal the total phenols. Whether these results were verified by other tests on these specimens or not, I do not know. In my own series of cases I found several times that the amount of free phenol equalled or exceeded the total phenols. However, carefully repeated experiments on these same specimens showed that in the process of heating with HCl a part of the phenol had escaped by evaporation.

Nevertheless Folin and Denis' general average of 89 per cent of free phenol and my own of 83 per cent show what a remarkably large percentage of the phenols is excreted in the free form. This, too, in spite of the fact that the latest text books on biochemistry (Hammersten, Matthews, Hawk, and Neubauer and Huppert) so far as they refer to this point at all, state the very opposite.

To show that the number of stools per day has an effect on the excretion of phenols, as Folin believed and as Salkowski[10], Brieger[8], Russo[13], and Strasser[12], have shown, I have gone over the clinical charts available on my cases and tabulated the findings in Table 4.

Phenol excretion with reference to number of stools per day.

		First week		Second week		Third week	
No.	Change in weight during investigation. gm.	Number of stools	Total phenols mg.	Number of stools	Total phenols mg.	Number of stools	Total phenols mg.
6	-360	2.	35.6	5.1	31.2	1.3	29.1
7	+295	5.1	12.8	4.2	12.8	4.	12.
8	+600	4.	29.5	2.	41.8	5.4	22.3
9	+200	3.3	39.1	6.3	27.1	3.6	24.9
10	0	7.	17.6				
20	- 50	1.	33.8				
11	+ 90	7.	10.5				
12	+ 30	3.	22.7	4.	21.1		
13	+ 40	2.	13.6				
14	+ 80	1.3	9.0				
18	+120	1.5	17.0				
21	+210	2.	36.7				
22	- 70	2.	21.6				

If one considers three stools per day to be normal for infants, then, of the twenty-two comparisons here shown, ten are normal and twelve abnormal. The average excretion of phenols by those having

3 stools or less per day = 26.1 mg.

over 3 stools per day = 21.7 mg.

If, however, one compares those having four or more stools with those having three or less, the results are more striking.

3 stools of less per day = 26.1 mg. phenol

4 stools or more per day = 16.2 mg. phenol

A comparison of cases 10 and 20, two children of the same age, whose total nitrogen excretions are 1.5 and 1.2 gm. respectively, apparently furnishes a striking example of the effect of diarrhea upon phenol excretions. Whether this is due entirely to the enteral conditions or partly to par-enteral metabolism can hardly be considered a settled question.

Changes in weight have no demonstrable effect.

For the purpose of a further metabolic study with reference especially to artificial and breast feeding, I have summarized in Table 5 the more important factors.

Table 5. A comparison of diets.

No.	Age mos.	Weight Kilos.	Urine cc.	Total phenol mg.	Total nitrogen gm.
Artificial milk.					
4	3	4.3	705	58	2.890
5	6	3.6	726	55.8	1.260
6	6	5.1	781	30.9	1.917
8	4	3.5	698	31.9	1.303
9	4	4.4	605	30.3	1.526
10	6	5.3	799	24.0	1.528
12	2	4.7	583	21.9	1.686
20	6	4.9	210	33.8	1.192
18	3	3.7	225	17.0	1.185
Average	4.1	4.4	591	33.7	1.609
Breast milk.					
7	2	3.2	420	12.6	.426
11	1.25	1.8	465	10.5	.382
13	.5	2.9	150	13.6	.264
14	.25	2.9	34	9.0	.293
21	1.50	5.9	402	36.7	.412
22	2.	2.8	155	21.6	.358
Average	1.25	3.3	271	17.3	.356

The artificially fed infants show a phenol excretion of 8 mg. per kilogram of body weight; breast fed, 5 mg. per kilo. Meyer[27] does not give the weights of all his infants. Nevertheless he observed that "the decomposition in those fed on cow's milk allowed the formation of more phenol than in those fed on breast milk."

In so limited a number of cases any proportions must be ascribed partly to accident. In my cases the artificially fed compared with the breast fed show a phenol-excretion ratio of 2:1; in Meyer's cases 3:1. Unfortunately Meyer does not give the total nitrogen excretion in his cases so we do not know how his and our results compare in the light of protein intake.

That age and consequent increase of protein in the diet, as evidenced by total nitrogen excretion, have a decided effect on phenol excretion is indicated in the following averages:

Age	Total N. gm.	Total Phenols mg.
4-6 months (6 cases)	1.454	34.3
1-3 months (7 cases)	.763	25.5

It is also noteworthy that, without reference to age, an increase in total nitrogen is accompanied by an increase in total phenols. A working rule which might be formulated from these cases is: trebling the protein intake doubles the phenol excretion.

The nitrogen-phenol ratio in adults is interestingly shown by summarizing the cases reported by Folin and Denis[29] as follows:

Diet	Total N. gm.	Total Phenols gm.
High protein (14 cases)	18.76	.443
Low protein (16 cases)	5.98	.240

These 30 cases were the usual run of city hospital patients including typhoid, pernicious anemia, carcinoma, pleurisy, and three cases of pneumonia with temperatures of 103°- 104°.

They also compared high and low protein diets in the same individual on each of three cases with the following average results:

Diet	Total N. gm.	Total Phenols. gm.
High protein	19.2	.428
Low protein	6.7	.215

It is to be noted that this rule worked out on infants,- trebling the protein doubles the phenols - applies to adults both collectively and individually.

In comparing the results of Folin and Denis in their experiments on infants (Table 3) with my own (Table 1) and applying the rule given above, we find an explanation for their average of 48 mg. as compared with mine of 29 mg.

total N. : total N. :: phenol : phenol

1.74 : 1.107 :: 48 : x (=30)

If it be true that the excretions of nitrogen and phenols bear a definite relation both in infancy and adult life without reference to disease, weight, length of bowel, or number of stools, may it not be true that the phenols in the urine are also in part at least a product of endogenous metabolism?

Blumenthal and Lewin[30] were the first to advance this view. They based their belief upon an increased phenol excretion in cachetic cancer and increased protein katabolism following phloridzin injection. Mayer[31] and Scholz[32] were unable to verify these results in animal experiments after phloridzin injection.

A. Ellinger[33] accepts this and the statement of Senator[26] that "none or only traces of phenol are present in the urine of newborns" because "in them the bowel content is sterile" (Schild[34]), as sufficient proof that phenol is formed only through bacterial action within the bowel.

Senator[26] tested the urine, liquor amnii, and meconium using Baumann's distillation method[5] and testing the distillate with Millon's reagent. The conjugated sulphates (phenol and cresol) he converted into tribromphenol and tribromcresol.[5] In testing the urine he used an average of 33 cc. which must have required from one to three days to collect. Out of five tests on the urine only two were positive. The liquor amnii and meconium were both negative, as were also the stools until the children were a month or more of age.

The question of the origin of phenols seemed therefore to rest largely upon their presence or absence in the urine of newborns.

In an attempt to answer this question by the Folin and Denis method we collected urines at the University of Minnesota hospital from newborns during the first three days of life. The infants were treated according to the usual hospital routine, being put to the breast every four hours after the first half day. Only two cases (30 and 31) received any water during the three days and those only 15 cc. each. The specimens were collected from male babies in the winter time and were preserved with chloroform and kept on ice.

A total of 19 cases were studied with results as given in Table 6.

TABLE #6

NEWBORNS

No. Name.	Clinical Condition	Age in hours at start of 72 hrs spec.	Weight at birth with change in the 72 hrs.	Stools	Temperature range in the 72 hrs.	Urine: Volume cc.	Urine: Specific Gravity	Urine: Total Nitrogen mg.	Phenols: Free mg.	Phenols: Total mg.	Phenols: % Free	Phenols: % Conjugated	
#23 Lepisto	Pemphigus.	8 hrs.	2710 −250	2, meconium	98-100.6	48	1022	320	7.9	10.3	76	24	
#24 Schonning	Normal.	36	4215 +60	2,mec 1, L	98.6-99.4	93	1026	1427	16.7	20.2	82	18	
#25 Lohn.	Normal.	27	3365 −5	2,mec 1, L	98-99.2	60	1018	400	12.8	20.6	62	38	
#26. Ranta.	Icterus, Pemphigus.	3.	3725 −585	3,mec	98.6-103.6	47	1027	382	15.6	19.9	77	23	maybe 4 hr. spec. only
#27. Rester.	Icterus.	38.	4090 −250	1,mec 1, L	98.6-100	69	1024	853	15.4	23.4	66	34	
#28. Bromberg	Perioral Dermatitis.	1	3505 −310	2,mec 1, L	98.6-100.4	54	1022	481	14.1	15.1	93	7	
#29 Tion.	Icterus.	12	2945 −40	2,mec	97.8-98.4	36	1018	147	7.8	8.3	85	15	
#30 Hatcher	Icterus.	17	2990 −225	2,mec 1, L	97.4-99.	31	1026	157	4.3	5.1	84	16	Received 15 cc. water 3 hrs. before spec. started.
#31. Svander.	Normal.	22	3355 −260	2,mec 1, L	97.2-99.2	55	1024	610	12.1	13.4	90	10	Received 15 cc. water 4 hrs. before spec. started.
#32. Jorgensen.	Icterus.	.5	3630 −440	2,mec	97.2-100.2	14	—	94.	1.2	2.9	41	59	
#33. Anderson.	Icterus.	1.	4115 −325	2,mec	97.6-99.	34	—	275	11.8	14.6	81	19	
#35. Anderson	Icterus.	1.	3585 −440	3,mec	97.2-99.6	25	—	127	7.4	8.5	87	13	
#36. Moore.	Icterus.	0	3690 −290	1,mec	98.8-100.	11	—	39	1.8	2.1	86	14	
#37. Anderson.	Normal.	.5	2805 −265	2,mec	97-101	23	—	198	4.6	5.7	80	20	
#38. Peterson.	Normal.	2.	3650 −310	3,mec	98-98.6	43	1006	301	7.2	8.8	82	18	
#39 Conklin.	Normal.	0.	3025	2,mec	96.4-98	33	1011	199	4.6	6.0	77	23	
#40 Rohl.	Male Cephalhematoma.	.5	4630 −250 (in 48 hrs)	0	96.4-97.6	70	1016	491	12.1	15.1	80	20	Died at 76 hrs. of sub-dural hemorrhage
#41. Arkell.	Icterus.	1.	3275 −255	3,mec	96.2-100	7	—	—	2.4	34	70	30	
#42 Olson.	Normal.	2	4215 −475	1,mec 1, L	96.6-100.8	34	1016	280	8.6	9.9	86	14	
	Average.		3655			41		376	8.7	11.2	76	24	

Phenol was present in every case, the amounts varying from 2.1 to 23.4 mg. The average urine volume for three days was 41 cc., the average loss of weight 260 mg. If we use the weight at the end of the three days as a basis, the excretion per kilogram is 3.4 mg. This seems remarkable when we consider that this is practically a period of starvation.

The average total phenol was 11.2 mg., of which 76 per cent was free and 24 per cent conjugated. The total nitrogen averaged 376 mg. Furthermore, compared with infants, the phenols and total nitrogen are both low. If we apply the nitrogen-phenol rule to these cases we find, 1.74 : .376 :: 48 : x (10.4) The computed phenol result (10.4) approximates the obtained result (11.2) closely enough to indicate that, when taken collectively, the nitrogen-phenol ratio in fasting newborns is the same as in infants and adults.

Summary.

The Folin and Denis method of determining total phenols in the urine gives more uniform results in cold weather when modified as here suggested.

An increase in the number of stools per day is associated with a decrease in the total phenol output.

In this series of fifteen infants the phenol excretion per kilogram of body weight is less in the breast fed than in the artificially fed.

In infants and in adults, there is an interrelation between the total phenol excretion and the protein intake as measured by the total nitrogen excreted in the urine. Trebling the proteins doubles the phenols expresses, in general terms, this

ratio so far as it is possible from the limited number of cases now at hand.

Contrary to the findings of Senator, phenols are quantitatively present in the urine of every newborn. The average of these 19 cases is 11.2 mg.

As phenols are present during this period of starvation they must originate in part through endogenous metabolism.

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