STUDIES ON THE METABOLISM OF HYDROQUINONE

THESIS FOR THE DEGREE OF M. S.

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Hydroquinone

Chemistry

STUDIES ON THE METABOLISM OF HYDROQUINONE

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By

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INTRODUCTION

Work has been done in this laboratory and elsewhere (1, 2, unpublished data) showing that hydroquinone, when added in small quantities, will prevent the oxidation of certain readily oxidizable foods. It was found that certain foods could be stored for long periods without undergoing oxidative change, if they were first thoroughly mixed with a small quantity of hydroquinone. It has also been shown that this chemical will prevent the destruction of vitamine A. The commercial use of hydroquinone in this role as a preservative would supplant at least in some cases the present bothersome and expensive methods of preservation, such as vacuum packing. In view of this it was thought advisable to undertake a study of the fate of hydroquinone in the animal body.

No previous work on this subject has been reported in the literature. There have, however, been reports on several closely allied compounds, benzene (3,4,5,6,7,8,9,), halogen derivatives of benzene (9,10,11), phenol (10,11,12), benzoic acid (13), acetophenone (6), and fatty aromatic compounds (14).

The results of these researches may very well have some bearing on the problem at hand. Thus, it has been found that when benzene is fed or injected, hydroquinone and pyrocatechol may be eliminated in the urine

in amounts large enough to permit isolation and identification (15). Preusse (16) found that paracresol when fed to dogs is in part excreted as paracresol (ester) and is in part oxidized to paraoxybenzoic acid.

Jaffé (8) found that upon administering benzene it was possible to isolate the straight chain muconic acid from the urine, indicating that the benzene nucleus had been split. He fed 60 grams of benzene in lots of 3 grams per day and recovered approximately 3% of this as muconic acid. He could isolate no muconic acid when benzene was not fed. Upon injection of 8 grams of muconic acid as the sodium salt subcutaneously in four doses in the course of 12 hours, only 1% was recovered in the urine. He believed this to show that muconic acid itself was readily oxidized by the animal body. Fuchs and v. Soós (4) also found that when 3 - 5 grams of benzene was administered daily to leukemia patients, muconic acid could be isolated from the urine.

Mori (17) contrary to the results of Jaffé, found that a large portion of the muconic and adipic acids administered to dogs was excreted unchanged in the urine. When injected subcutaneously, from 71.4% to 74.1% of the muconic acid was recovered in the urine. When given by way of the stomach, 43.6% was recovered. Neumaerker (3) was also unable to duplicate the work of Jaffé and Fuchs and v. Soòs. He injected benzene in

doses of not more than 3 grams per week and could isolate no muconic acid. Upon injection of 4 grams of muconic acid he recovered from 55.1% to 67% of it unchanged in the urine. He concluded in accordance with Mori and contrary to Jaffé, that muconic acid was not easily oxidised in the body.

Thierfelder and Klenk (6), however, correlated the above results, showing that if sufficient benzene were fed or injected and the absorption into the body were rapid, muconic acid could be isolated in the urine. They believed the differences in muconic acid oxidation found by Jaffé as against Neumaerker and Mori to be due to concentration of the solution injected, differences in the weights of the experimental animals as well as individual differences of the animals themselves.

Underhill and Harris (5) reported that benzene "acts not only on the blood elements but exerts a catabolic influence on the body tissues as a whole, as manifested by a sharp rise in creatinine and total nitrogen within a very short period after its subcutaneous injection".

Several investigators have found that sulphur metabolism was disturbed by administration of benzene or its derivatives. Callow and Hele (9,18) found that upon feeding mono- and di-chlorobenzene they were excreted in part as chloro-phenylmercapturic acid and in part as $C_6 H_4 Cl \cdot HSO_4$. They found that this caused an

increase in the S/N ratio of the urine. This they explained by suggesting that the sulphur metabolism was hastened and the nitrogen of the catabolized protein was excerted later. Toluene and o-chloro toluene showed no such effects.

Rhode (11) administered (.2 g. per kg.) phenol simultaneously with cystine, taurine and Na_2SO_3. The percentages excreted as ethereal sulfates were 33%, 17% and 27% respectively. Inorganic sulfates and thio-sulfates were without apparent effect. When bromo-benzene and di-bromo-benzene were fed they appeared in the urine partly as ethereal sulfates, but when cystine was given simultaneously they appeared as mercapturic acid.

Shiple, Muldoon and Sherwin (10) found that a pig reduced to a condition of endogenous N catabolism and maintained on a carbohydrate diet, excreted about 4 mg. of ethereal sulfates per day. The animal was then fed C_6H_5Br, C_6H_5OH and p - C_6H_4OH Cl. The output of ethereal sulfates was very decidedly increased in each case, evidencing the formation of ethereal sulfates from endogenous sources. The feeding of inorganic sulfates along with each of the toxic substances resulted in no increase in the elimination of sulfates. Cystine, however, together with each of the same aromatic poisons, caused an increase in the excretion of this form of sulfur with C_6H_5OH, but a decrease with the other two.

Moreover, with C_6H_5 Br. the decrease was accompanied by a corresponding rise in neutral sulfur. They concluded that there were two ways of detoxicating phenolic substances; one, by combining the poison with a sulfate radical, which is obtained by tissue destruction; the other by utilizing exogenous cystine, forming eventually a mercapturic acid. This mercapturic acid may be excreted as such, thereby adding to the neutral sulfur fraction and lessening that of ethereal sulfates, or it may be oxidized to a sulfate and increase the output of ethereal sulfates.

Folin and Denis (19) reported that the distribution of phenols between the free and conjugated forms is virtually the same in animals and man, the free phenols representing from 80% to 90% of the total. They also reported (20) that the amount of phenol excreted in the feces is so small as to be negligible. Dubin (21) in repeating the work of Folin and Denis corroborated these results. He also found phenols to be increased in the urine and the ratio of combined phenol to free phenol to be increased following withdrawal of water, intestinal obstruction or pancreatic insufficiency. After feeding 1 gm. of Ph OH or p-cresol to dogs weighing about 10 kilos. about 65% and 40% respectively were eliminated as phenols in the urine.

These reports on benzene and its derivatives,

although not directly applicable to the problem at hand, gave an idea as to what one might expect upon feeding hydroquinone. At the same time they indicated the direction in which to proceed with this problem.

EXPERIMENTAL

A pig was selected as the experimental animal with the idea in mind that the metabolism of swine approaches that of humans more closely than does the metabolism of other animals. The animal selected was a young pig of somewhat less than 100 pounds weight.

A cage was made for the metabolism work, consisting of two parts, the cage proper, and the feeding cage. The cage proper was 4' x 4' x 5' in dimensions. The cage was completely zinc lined. One side of it was hinged to permit easy access for cleaning. The top of the cage was covered with iron bars spaced about 6" apart. The cage was floored with heavy iron screen. The cage was on casters and was placed on a platform about a foot and a half in height. The platform was twice the length of the cage, one half of the platform consisting of a drain board covered with metal. This drain slanted at the same angle from each side, and at the center there was an opening under which a bottle was placed for the collection of urine. The cage stood over

the drain except when being cleaned. It could then be run from over the drain and the drain could be washed and scrubbed. The feeding cage was 1½' x 4' x 5' in size. The sides of this cage were also zinc lined and it was roofed with iron rods. The feeding trough, zinc lined, was placed at one end of the cage. A small door opened immediately above the trough to permit mixing the food. The floor of the back part of the cage consisted of heavy metal screen. Under this was a zinc-lined drawer to receive any urine voided while the animal was in the feeding cage. The two cages were connected by doors which could be raised or lowered at will. The cages were securely fastened to each other by hooks.

The diet selected for the experimental animal was a balanced ration made up as follows: 75% corn meal, 10% whole milk powder, 10% oil meal, 3% alfalfa meal, 1% Na Cl and 1% bone ash. On this diet the daily output of phenols remained fairly constant. The animal received 500 gms. of the ration per day. It was fed by mixing well with water in the trough. The hydroquinone was given with the food by dissolving it in the water added to the food mixture.

Animal I was placed on the above diet for two weeks before hydroquinone was fed. Phenols were determined daily on five consecutive days of each week during the

experiment. The question arose as to what method should be used in the determination of phenols. The method of Folin and Denis (28,20,19,29,30,31,32,33,34,35) was selected in spite of the fact that a number of investigators have found it to be non-specific (22,23, 24,25,26,27). A careful review of the literature failed to reveal another method which would adopt itself to daily routine. A very good review of the literature of phenol determinations up to the year 1926 is given by Gibbs in Chemical Reviews (36).

The results of these analyses are given in Table I. It will be seen, taking the figures of the first two weeks as a basis, that a little more than three-fifths of the hydroquinone was excreted daily as fed. This appeared in the urine both as free and conjugated phenol in about normal proportions. During the sixth week when 3 gms. of hydroquinone was fed daily the proportion of conjugated phenols was increased slightly. This might indicate a special power of detoxication in the body in the presence of extraordinarily large quantities of phenolic substances. After discontinuing the feeding of hydroquinone it will be noted that the phenol content of the urine did not return at once to normal. The phenol excretion for the first three weeks after the administration of hydroquinone was stopped was definitely higher than that of the two weeks control period

preceding the feeding of hydroquinone. There was a gradual decrease towards the normal values, however. Animal I differed from all the ensuing animals in this respect.

These data would seem to show that the greater portion of the hydroquinone was excreted as fed. Apparently, however, some of the material was stored in the body and was excreted after the feeding of hydroquinone was discontinued. Using the control period as a basis, approximately 75% of the hydroquinone was excreted as free and conjugated "phenols". The animal showed no apparent detrimental effects as the result of this experiment.

TABLE I

	Free phenol per day	Total phenol per day	% conj. phenol	
Apr. 22	670.5	957.6	29.98	
Apr. 23	253.26	311.6	18.74	no hydro-
Apr. 24	650.6	897.5	27.50	quinone
Apr. 25	381.4	536.9	28.95	
	1,955.9	2,703.9	27.66	
Apr. 28	191.3	340.9	43.87	
Apr. 29	336.5	495.5	32.09	no hydro-
Apr. 30	240.6	316.3	23.92	quinone
May 1	253.9	371.04	28.22	
May 2	267.8	341.7	21.63	
	1,333.8	1,865.5	28.49	
May 6	515.4	818.9	37.30	
May 7	856.8	1,364.5	38.72	1 gm. hydro-
May 8	655.6	1,111.08	40.98	quinone daily
May 9	303.6	395.3	23.19	
May 10	860.8	1,264.6	31.92	
	3,170.4	4,954.5	36.04	
May 12	659.5	1,081.5	39.004	
May 13	530.2	930.7	43.02	1 gm. hydro-
May 14	742.2	1,147.4	36.18	quinone daily
May 15	1,364.1	1,661.1	17.88	
May 16	610.03	752.7	19.85	
	3,907.1	5,573.4	29.53	
May 19	1,373.5	2,499.8	45.05	
May 20	401.3	619.5	35.21	2 gms. hydro-
May 21	704.4	1,263.5	44.22	quinone daily
May 22	957.3	1,468.9	34.82	
May 23	1,608.8	2,160.0	25.51	
	5,045.5	8,011.8	37.02	
May 27	915.6	1,774.9	48.41	
May 28	1,246.8	2,625.02	52.5	5 gms. hydro-
May 29	1,082.2	2,378.1	54.55	quinone daily
May 30	947.0	1,883.7	49.72	
May 31	876.0	1,890.7	53.63	
	5,067.0	10,542.6	51.93	

TABLE I (Con't).

	Free phenol per day	Total phenol per day	% conj. phenol	
June 3	1,580.5	1,844.5	14.31	
June 4	436.7	732.3	40.37	no hydro-
June 5	656.6	1,069.6	38.71	quinone
June 6	541.2	868.7	37.70	
June 7	593.4	1,140.4	47.96	
	3,808.4	5,655.8	32.60	
June 9	917.9	1,302.7	29.52	
June 10	401.2	566.08	29.11	
June 11	635.2	840.8	24.44	
June 12	554.4	651.8	14.94	
June 13	524.5	640.8	18.17	
	3,033.2	4,002.2	24.21	
June 16	601.2	773.7	22.30	
June 17	562.4	744.6	24.47	
June 18	574.5	711.7	19.28	
June 19	525.00	692.6	24.20	
	2,263.17	2,922.8	22.41	
1st week	1,955.9	2,703.9	27.66)	no hydro-
2nd week	1,333.8	1,865.5	28.49)	quinone
3rd week	3,170.4	4,954.5	36.04)	1 gm.
4th week	3,907.1	5,575.4	29.53)	daily
5th week	5,045.5	8,011.8	37.02)	2 gm. daily
6th week	5,067.0	10,542.6	51.93)	3 gm. daily
7th week	3,808.4	5,655.8	32.6)	no hydro-
8th week	3,033.2	4,002.2	24.21)	quinone
9th week	2,263.17	2,922.8	22.41)	

In the case of Animal II, the same experimental procedure was followed as with Animal I, with the exception that creatinine was also determined daily. The results, however, were somewhat different as may be seen in Table II.

In this case there was a marked increase in the conjugation of urinary phenols as soon as the administration of hydroquinone was started. The percent of conjugation did not increase further, however, when the amount of hydroquinone given was increased. With Animal II, using the two week control period as a basis for calculation, approximately 25% of the hydroquinone fed was excreted as free or conjugated "phenol". As soon as the feeding of hydroquinone was stopped the urinary phenols returned at once to normal values and the percent of conjugation dropped to the values obtained before the administration of hydroquinone.

The creatinine excretion remained fairly constant throughout the experiment. The pig seemed to develop normally and no deleterious effects of the experiment could be noticed. This animal was approximately the same size as the preceding one.

TABLE II

	Free phenol per day	Total phenol per day	% conjugation	Creatinine	
Oct. 21	464.4	695.2	33.2	997.3	
Oct. 22	682.4	780.5	12.5	1,085.0	no
Oct. 23	565.1	765.3	26.1	981.5	hydro-
Oct. 24	523.9	667.8	21.5	1,029.3	quinone
	2,235.8	2,908.8	23.3	4,093.1	
Oct. 27	440.9	496.6	11.2	1,901.9	
Oct. 28	765.6	829.3	7.6	1,050.3	no
Oct. 29	619.2	651.8	4.9	1,061.7	hydro-
Oct. 30	316.4	391.5	19.1	999.9	quinone
Oct. 31	459.3	503.7	8.8	908.06	
	2,601.4	2,872.9	10.3	5,901.86	
Nov. 3	806.4	1,007.02	19.9	1,203.1	
Nov. 4	584.7	853.1	31.4	1,158.7	1 gm.
Nov. 5	575.5	924.1	37.7	1,617.8	daily
Nov. 6	551.6	969.6	43.1	1,009.3	
Nov. 7	390.05	681.5	42.7	841.02	
	2,908.2	4,435.3	34.9	5,829.92	
Nov. 10	774.9	1,076.8	27.9	1,589.1	
Nov. 11	619.1	886.1	30.1	1,141.4	1 gm.
Nov. 12	485.08	751.7	35.4	796.6	daily
Nov. 13	595.08	718.8	17.2	1,168.1	
Nov. 14	615.7	858.7	28.2	661.6	
	3,089.8	5,291.1	27.7	5,296.8	
Nov. 17	580.1	865.9	33.05	1,210.5	
Nov. 18	688.7	895.5	11.9	1,190.2	1 gm.
Nov. 19	644.6	985.5	35.5	1,042.5	daily
Nov. 20	659.4	989.05	51.1	1,115.6	
Nov. 21	680.9	1,065.9	36.002	1,613.9	
	3,253.7	4,839.4	29.5	6,173.0	
Nov. 24	818.5	1,431.1	42.8	1,038.9	
Nov. 25	590.08	1,036.007	42.07	1,096.9	2 gms.
Nov. 26	389.1	726.2	46.19	879.8	daily
Nov. 27	950.6	1,340.7	30.5	1,233.7	
Nov. 28	527.7	801.1	34.05	---	
	3,25417	5,332.1	39.6	5,249.3	

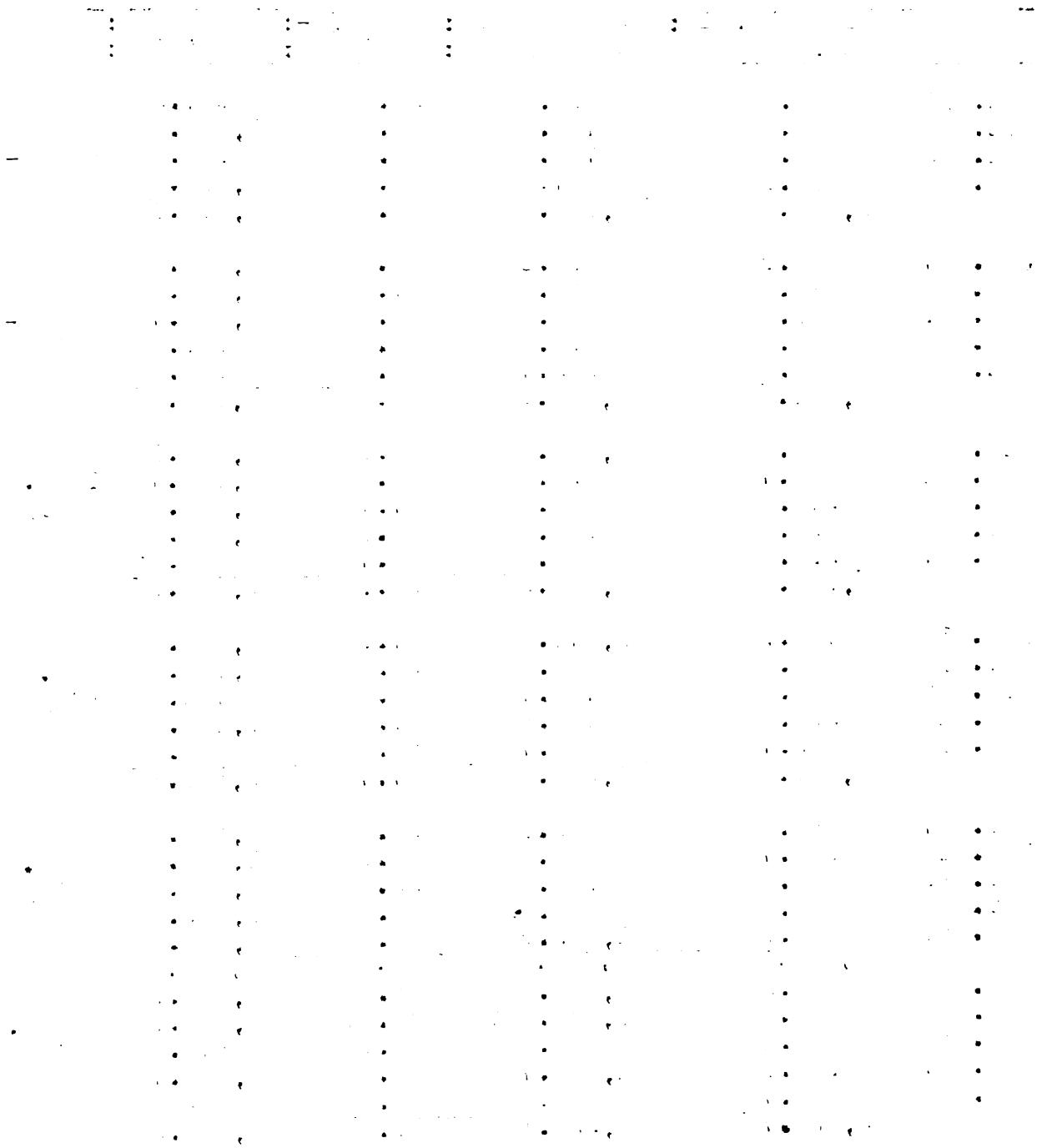
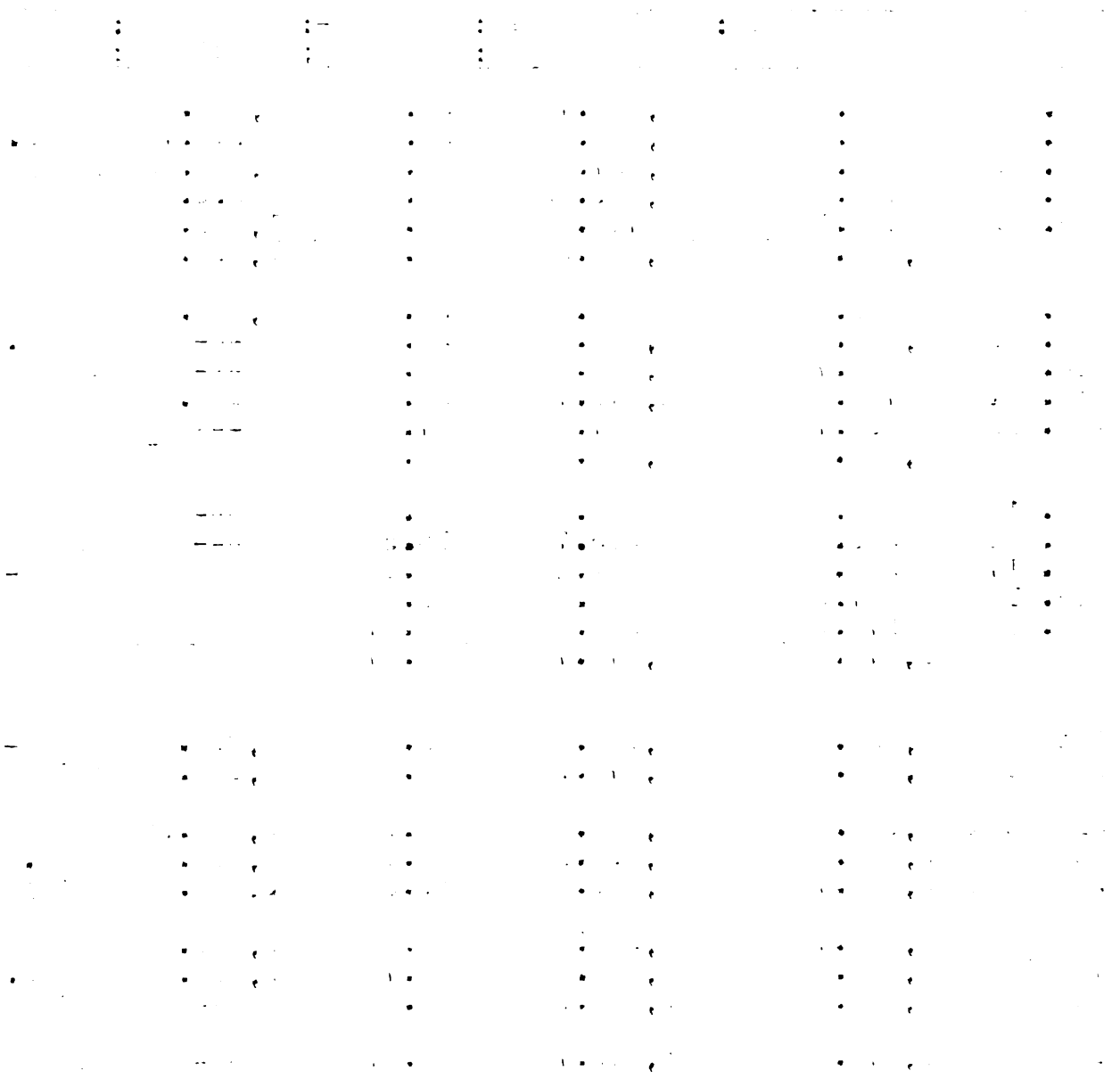


TABLE II (Con't)

	Free phenol per day	Total phenol per day	% conjugation	Creatinine	
Dec. 1	851.8	1,269.7	34.48	1,308.5	
Dec. 2	724.8	1,048.1	30.8	974.7	2 gms.
Dec. 3	884.6	1,237.5	28.5	1,202.05	daily
Dec. 4	798.5	1,019.05	21.1	878.5	
Dec. 5	743.1	771.06	3.6	1,031.5	
	3,982.8	5,345.5	25.7	5,395.5	
Dec. 8	549.8	826.5	33.4	1,026.5	
Dec. 9	1,081.0	1,621.5	33.33	---	2 gms.
Dec. 10	851.7	1,261.9	34.8	---	daily
Dec. 11	729.2	1,079.7	32.4	919.8	
Dec. 12	694.7	957.4	27.4	---	
	3,886.4	5,746.9	32.5		
Dec. 15	498.1	490.6	1.5	---	
Dec. 16	542.5	413.7	15.5	---	no
Dec. 17	344.4	361.1	4.6		hydro-
Dec. 18	417.5	425.4	1.4		quinone
Dec. 19	270.5	386.9	30.07		
	1,879.8	2,075.7	10.67		
					no
1st week	2,255.8	2,908.8	23.5	4,093.1)	hydro-
2nd week	2,601.4	2,872.9	10.5	5,901.86)	quinone
3rd week	2,908.2	4,435.5	34.9	5,829.92)	
4th week	3,089.8	3,591.1	27.7	5,296.8)	1 gm.
5th week	3,233.7	4,839.4	29.5	5,173.0)	daily
6th week	3,254.7	5,332.1	39.6	5,249.5)	
7th week	3,982.8	5,345.5	25.7	5,395.5)	2 gms.
8th week	3,886.4	5,146.9	32.5	---)	daily
9th week	1,879.8	2,075.7	10.67	---	no hydro- quinone



The experiment was repeated a third time, using the same experimental animal as in the preceding case. The results of this third trial, as given in Table III, parallel those of the second to a fair degree. Upon feeding hydroquinone there was an immediate increase in the excretion of both free and conjugated phenols. The percent of conjugation was also substantially increased. As in the preceding trial there was no further increase in the percent of conjugation upon increasing the amount of hydroquinone administered. When 1 gm. was fed daily about 56% of the hydroquinone was excreted as free or conjugated "phenol", and when 2 gms. were fed daily about 26% was excreted. Upon the cessation of administering hydroquinone the free phenol, total phenol and percent of conjugation immediately dropped to approximately the same values as before the experiment. In contrast to the preceding experiment, however, there seemed in this case to be a definite gradual increase in the creatinine excretion upon feeding hydroquinone. This reached a peak during the fifth week and from then on gradually returned to a normal value.

TABLE III

	Free phenol per day	Total phenol per day	% conjugation	Creatinine	
Jan. 13	625.00	727.9	14.4	1,088.05	no
Jan. 14	582.28	794.0	26.6	1,897.6	hydro-
Jan. 15	406.8	547.5	25.8	1,124.5	quinone
Jan. 16	446.9	594.6	24.8	1,250.7	
Jan. 17	321.2	404.6	20.6	1,206.4	
	2,382.4	3,068.7	22.06	5,947.25	
Jan. 20	489.2	593.9	17.62	1,344.9	no
Jan. 21	400.1	480.6	16.75	1,113.9	hydro-
Jan. 22	366.5	413.05	11.24	1,127.8	quinone
Jan. 23	337.2	406.8	17.11	1,020.9	
Jan. 24	282.8	358.7	21.14	1,269.8	
	1,876.1	2,253.2	16.7	5,877.3	
Jan. 27	358.5	578.7	38.06	1,021.89	
Jan. 28	676.7	959.9	29.47	1,384.61	1 gm.
Jan. 29	586.9	920.6	36.35	1,515.2	daily
Jan. 30	475.6	677.6	29.81	1,521.7	
Jan. 31	557.9	959.18	42.72	1,579.4	
	2,255.8	4,026.1	35.5	6,622.80	
Feb. 3	459.9	789.3	41.22	1,262.9	
Feb. 4	458.2	696.3	51.97	1,290.08	1 gm.
Feb. 5	692.0	1,197.9	42.24	1,956.4	daily
Feb. 6	700.7	1,044.3	32.9	1,583.5	
Feb. 7	323.0	505.6	36.11	1,083.6	
	2,613.9	4,233.9	40.9	7,176.4	
Feb. 10	666.5	1,055.4	36.94	1,407.3	
Feb. 11	678.7	1,113.8	39.06	1,884.6	1 gm.
Feb. 12	633.5	972.06	34.82	1,518.6	daily
Feb. 13	672.2	1,056.5	36.36	1,680.7	
Feb. 14	636.7	1,010.3	36.97	1,554.0	
	3,287.8	5,207.9	36.8	7,445.2	
Feb. 17	689.6	1,206.05	42.81	1,480.2	
Feb. 18	580.1	1,068.09	45.69	1,093.6	2 gm.
Feb. 19	601.2	1,087.36	44.8	1,359.2	daily
Feb. 20	650.6	1,108.00	41.27	1,084.3	
Feb. 21	759.3	1,173.40	35.28	1,075.8	
	3,280.8	5,642.9	41.9	6,099.1	

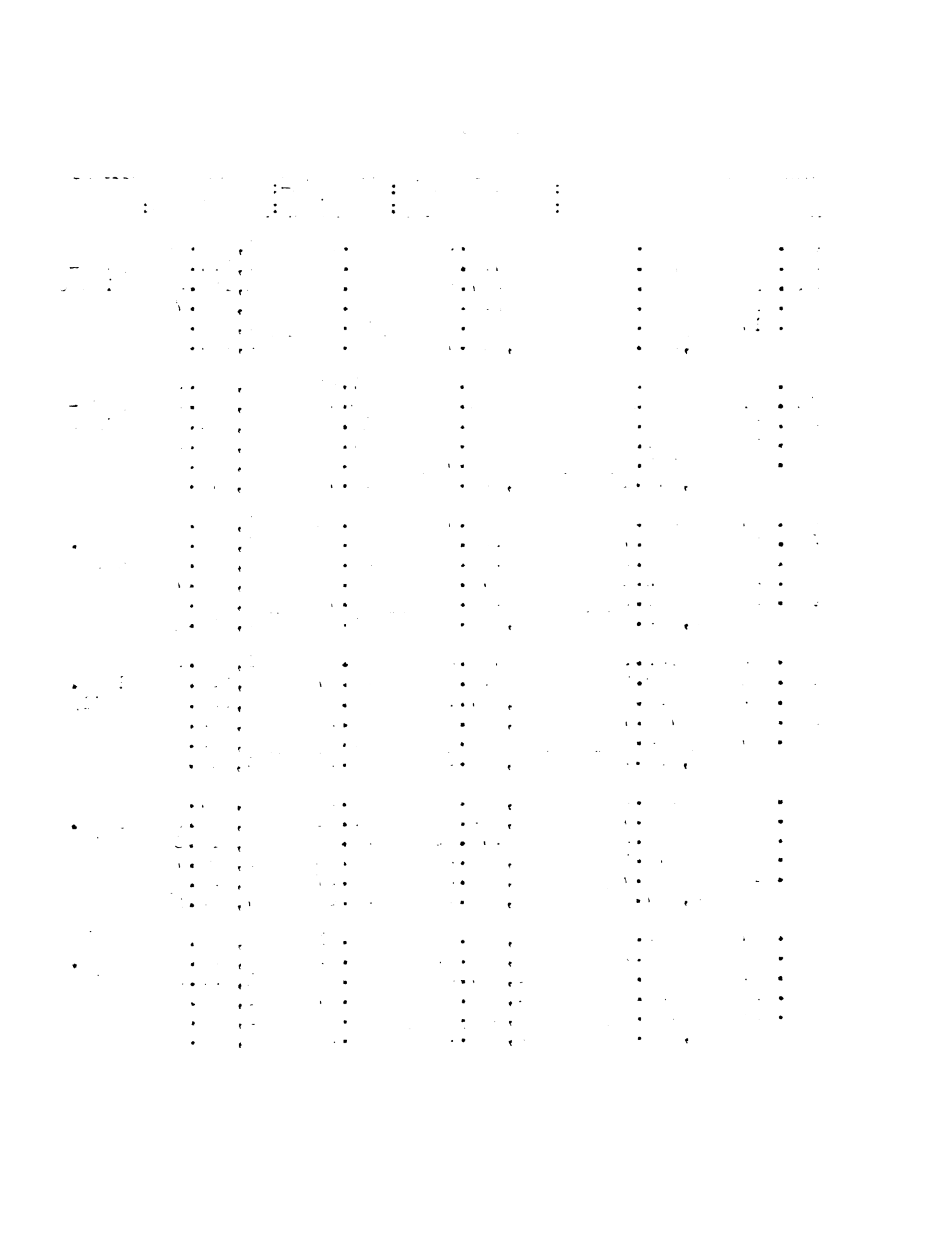
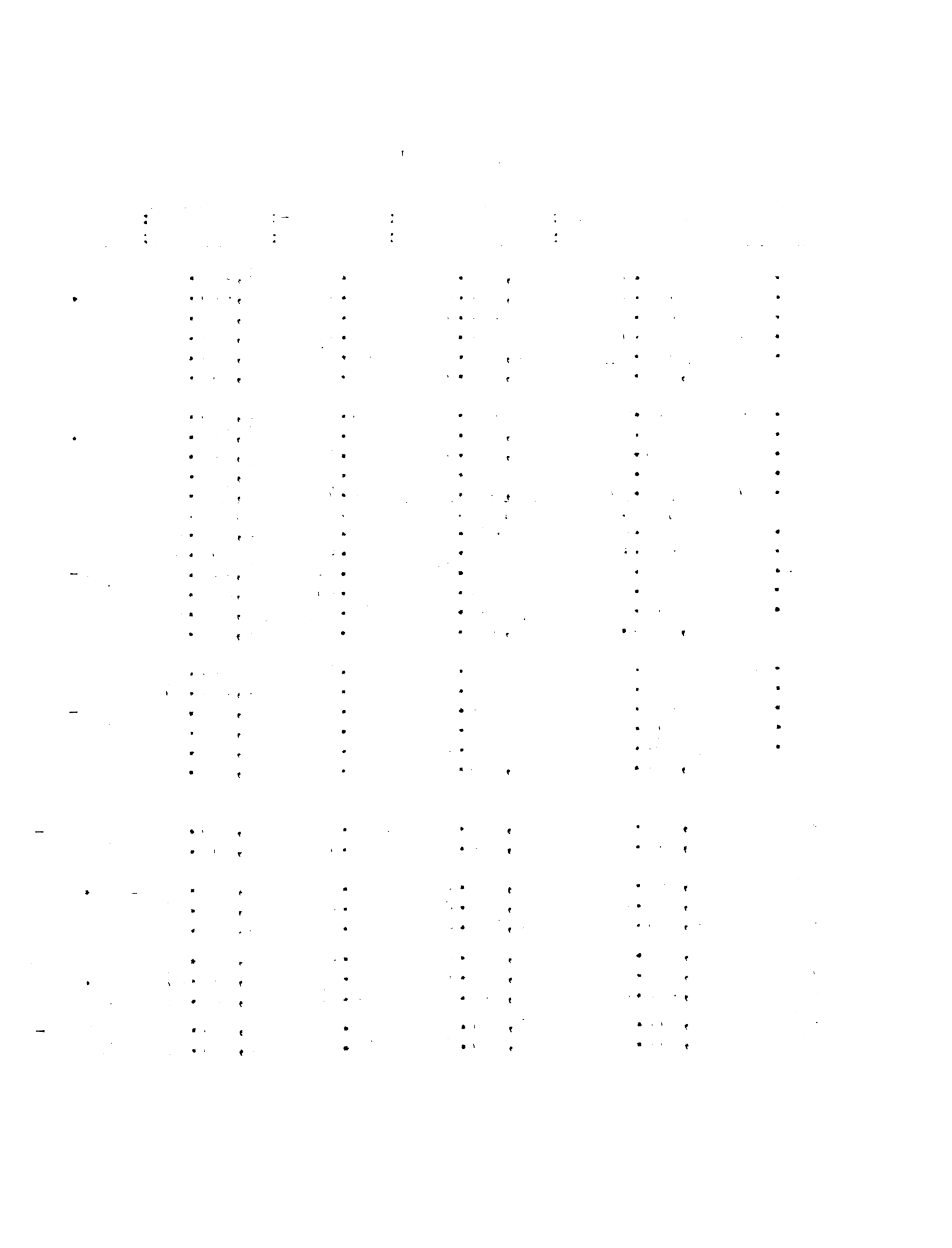


TABLE III (Con't)

	Free phenol per day	Total phenol per day	% conjugation	Creatinine	
Feb. 24	622.7	1,104.6	43.62	1,167.8	
Feb. 25	709.7	1,259.3	42.75	1,357.08	2 gms.
Feb. 26	561.1	959.7	41.54	1,268.6	daily
Feb. 27	455.7	859.2	53.79	1,365.5	
Feb. 28	703.4	1,059.8	34.61	1,219.2	
	3,050.4	5,322.7	39.2	6,378.1	
Mar. 3	491.82	945.88	47.86	1,067.8	
Mar. 4	668.68	1,048.32	36.21	1,298.6	2 gms.
Mar. 5	617.94	1,043.78	40.80	1,156.0	daily
Mar. 6	460.04	828.12	44.44	1,104.1	
Mar. 7	664.07	1,224.5	45.76	1,185.04	
	2,902.5	5,090.6	43.01	5,811.5	
Mar. 10	454.3	538.08	15.56	1,090.5	
Mar. 11	390.9	518.09	22.65	974.6	no
Mar. 12	458.6	571.02	23.01	1,218.6	hydro-
Mar. 13	364.3	489.5	25.57	1,106.4	quinone
Mar. 14	479.1	679.1	29.56	1,149.3	
	2, 127.3	2,795.8	23.2	5,569.4	
Mar. 17	474.8	561.4	15.28	859.5	
Mar. 18	454.4	625.5	27.11	1,169.07	no
Mar. 19	505.5	705.2	28.32	1,221.40	hydro-
Mar. 20	375.0	495.8	24.31	1,121.4	quinone
Mar. 21	369.1	500.9	26.91	1,096.09	
	2,179.002	2,887.09	24.3	5,447.46	
1st week	2,382.4	3,068.7	22.06	5,947.2)	no hydro-
2nd week	1,876.1	2,253.2	16.7	5,877.3)	quinone
3rd week	2,255.8	4,026.1	35.6	6,622.8)	1 gm.
4th week	2,613.9	4,233.9	40.9	7,176.4)	daily
5th week	3,287.8	5,207.9	36.8	7,445.2)	
6th week	3,280.8	5,642.9	41.9	6,099.1)	
7th week	3,050.4	5,322.7	39.2	6,378.1)	2 gm.
8th week	2,902.5	5,090.6	43.01	5,811.5)	daily
9th week	2,179.0	2,887.0	24.3	5,447.4)	no hydro-
10th week	2,179.0	2,887.0	24.3	5,447.4)	quinone



In repeating the experiment a fourth time a new experimental animal was obtained. It was impossible to obtain an animal as large as the two preceding ones had been. Animal III, weighed about 40 pounds at the beginning of the experiment. As well as repeating the previous work, inorganic sulfates, total sulfates and total nitrogen were determined.

The pig was growing rapidly during the course of the experiment. This would bring about a normal increase in all the substances determined which must be taken into consideration in analyzing the data. The data obtained in this fourth experiment (Table IV) differs quite radically from those of the three preceding ones. Upon feeding hydroquinone there was an increase in urinary phenols, but the increase was almost entirely free phenol. The data show that conjugation was almost negligible during all except the last two weeks of the period in which hydroquinone was fed. Between the period in which 1 gm. of hydroquinone was fed daily and that in which 2 gms. were fed daily no hydroquinone was given for a week. During this week urinary phenol fell back immediately to normal values, although there was absolutely no conjugation. The only explanation that can be offered for this absence of conjugation is that the capacity of a young rapidly growing animal for conjugation is probably very limited and needs to be developed.

The first week of the period in which two grams of hydroquinone were administered daily shows an abnormally large increase in phenol excretion. Using the preceding week, in which no hydroquinone was given, as a basis, there was practically complete elimination of the hydroquinone during this first week on two grams daily. The second and third weeks of this period, or the eighth and ninth weeks of the experiment, show values for phenol excretion more nearly parallel to those earlier in the experiment.

During the eighth and ninth weeks there began to be some conjugation of the phenols. This conjugation steadily increased to the end of the experiment, no decrease being shown when the administration of hydroquinone stopped. This might substantiate the idea that lack of conjugation earlier in the experiment was in some way connected with the age of the animal. Now that the animal had grown older, conjugation of phenols increased.

During the period when one gram of hydroquinone was fed daily approximately 80% of the hydroquinone fed was excreted as free phenol. During the period when two grams were administered daily, approximately 66% was excreted. This was also almost entirely free phenol.

As was to be expected there was a gradual increase in creatinine output during the course of the experiment. Apparently, however, when hydroquinone was first fed there was an abnormal increase in creatinine output. From this time on, the increase was again very gradual until the feeding of hydroquinone was stopped, when there was a slight decrease. The hydroquinone apparently had a definitely stimulating effect on creatinine elimination in Animal III.

The data obtained from the determination of inorganic and total sulfates are rather inconsistent. There was a gradual increase in both during the course of the experiment. This increase did not seem to be affected by the administration of hydroquinone and was probably due to the normal growth of the animal. Although the values obtained for percent of conjugation of sulfates were inconsistent, a definite increase in ethereal sulfates during the feeding of hydroquinone is indicated.

The data on total nitrogen are also somewhat difficult to determine. There was a definite increase in total nitrogen elimination when hydroquinone was first fed. During the three weeks in which 1 gm. of hydroquinone was given daily, the total nitrogen values returned to approximately normal, however. When the feeding of hydroquinone was discontinued for a week, total nitrogen excretion decreased enormously to a value about half

that of the normal. When the feeding of hydroquinone was resumed and 2 gms. were fed daily there was a great increase in total nitrogen output to a value about four times that of the preceding week. The total nitrogen values then continued to be high as compared with the two weeks control period, but they decreased gradually during the three weeks that 2 gms. were given daily. After stopping the feeding of hydroquinone, however, instead of dropping as in the sixth week, the total nitrogen values increased substantially.

These data seem to show that the total nitrogen was definitely increased in animal III upon feeding hydroquinone, but that some sort of adjustment was made upon continuation of administration of hydroquinone which permitted the values to return to normalcy.

The figures for creatinine and total nitrogen both seem to indicate that hydroquinone caused a certain amount of tissue destruction in animal III.

TABLE IV

	Free phenol per day	Total phenol per day	% conjugation	Creatinine	Inorganic Sulf.	Total Sulf.	Total Nitrogen	
Apr. 28	427.7	591.0	27.61	316.6	.7944	1.0261	1.48	
Apr. 29	223.2	304.9	26.8	344.3	.5760	.7200	1.04	no
Apr. 30	226.8	256.1	11.46	222.7	.7425	.8685	1.19	hydro-
May 1	231.7	257.3	9.95	136.1	.9678	.8277	.934	qui-
May 2	230.3	274.5	16.18	170.6	1.1299	1.1160	1.98	none
	1,336.7	1,683.8	18.4	1,290.3	4,2097	4.6522	6.56	
May 5	283.8	350.5	21.92	257.5	1.460	1.43	1.59	
May 6	311.8	323.6	3.65	283.2	1.36	1.48	1.90	no
May 7	421.3	353.7	---	280.3	1.65	1.800	1.938	hydro-
May 8	323.9	291.0	12.71	247.7	1.3995	1.7105	1.53	qui-
May 9	291.6	333.9	---	268.8	1.4784	1.7472	1.50	none
	1.632.4	1,652.7	7.6	1,337.5	7.33	8.16	8.45	
May 12	1,102.9	1,190.4	7.35	495.7	1.365	1.720	2.294	
May 13	1,114.5	1,057.1	---	420.4	1.0115	1.0914	2.786	1 gm.
May 14	1,119.0	1,132.2	1.16	539.9	1.2320	1.4052	---	daily
May 15	1,100.0	1,037.5	---	448.9	1.1616	1.6664	---	
May 16	1,011.8	1,002.1	---	536.9	1.5787	1.7892	---	
	5,448.2	5,419.3	1.7	2,441.8	6.331	7.65		
May 19	1,169.8	1,086.2	---	442.3	1.1315	1.5147	---	
May 20	1,049.8	994.7	---	532.2	1.5138	1.5876	---	1 gm.
May 21	1,131.3	1,192.4	5.12	551.5	.9178	.9631	---	daily
May 22	1,056.0	857.5	---	503.5	1.30009	1.3246	---	
May 23	1,231.8	1,196.4	---	564.2	1.0224	1.1076	1.580	
	5,638.7	5,327.2	1.02	2,593.7	5.8778	6.4801		
May 26	1,348.3	1,199.4	---	424.1	1.5664	1.9224	1.02	
May 27	1,224.5	1,017.0	---	527.5	1.1078	1.1680	1.12	1 gm.
May 28	1,292.8	1,253.6	---	529.6	.9599	2.3932	1.62	daily
May 29	1,293.4	1,293.4	---	509.9	1.6480	2.3108	2.60	
May 30	1,581.1	1,529.1	3.32	611.6	1.6882	2.8809	2.17	
	6,740.7	6,292.5	.66	2,602.7	6.9399	10.65	8.53	

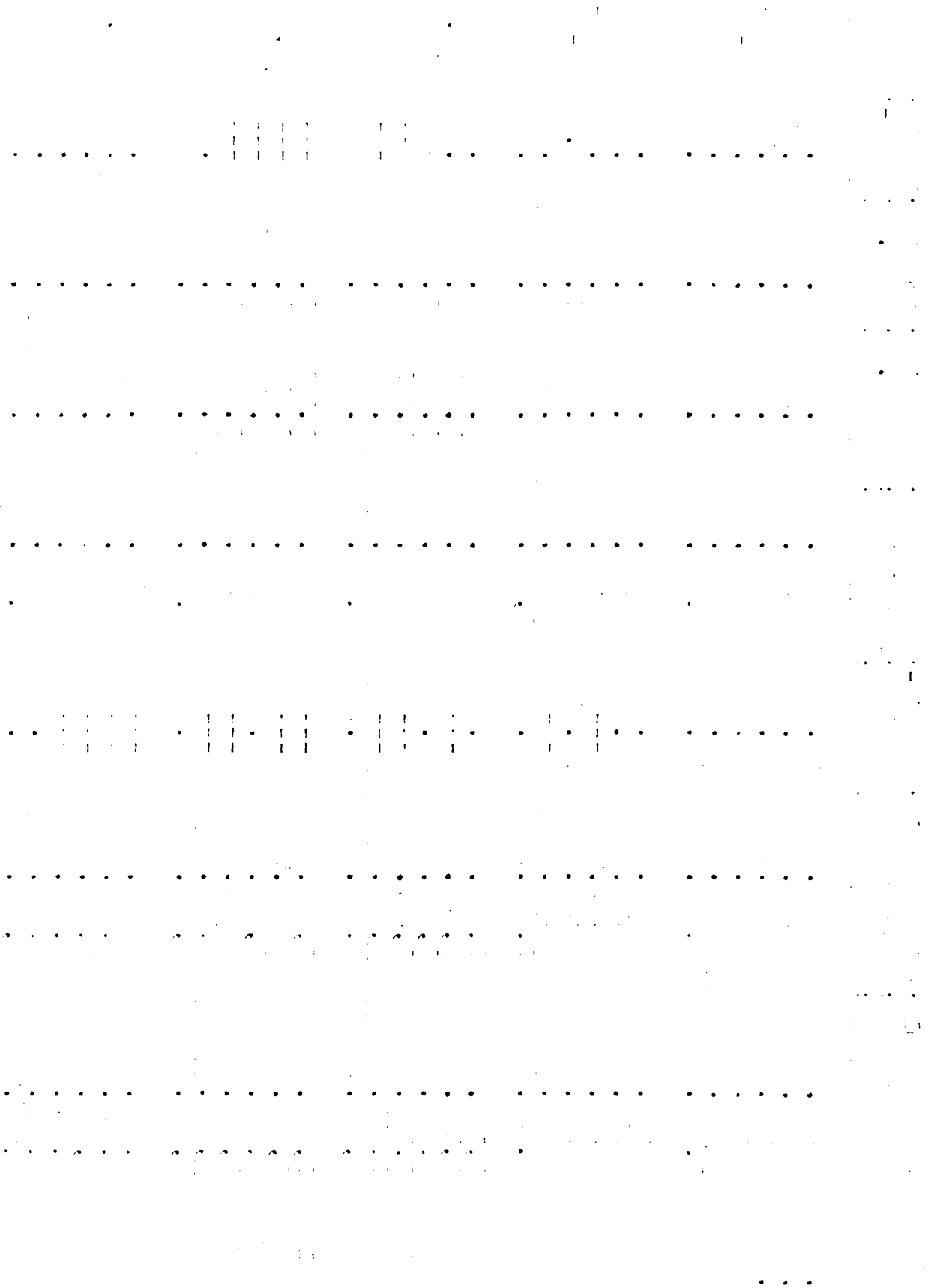


TABLE IV (Con't)

	Free phenol per day	Total phenol per day	% conjugation	Creatinine	Inorganic Sulf.	Total Sulf.	Total Nitrogen	
June 2	466.7	457.6	---	496.1	.5922	1.3076	.596	
June 3	487.5	489.5	---	542.6	1.989	2.223	.955	no
June 4	476.4	415.3	---	510.1	1.4742	1.9116	.557	hydro-
June 5	458.7	456.4	---	615.03	1.1421	2.3085	.904	qui-
June 6	447.2	427.0	---	654.2	2.3083	2.7480	.316	none
	2,336.5	2,245.8	---	2,818.03	7.482	10.47	3.33	
June 9	2,618.7	2,504.1	---	729.3	2.3614	2.5624	3.41	
June 10	2,439.7	2,210.7	---	698.4	1.8723	2.3416	2.23	
June 11	2,867.8	2,790.4	---	938.5	1.1357	1.3422	4.19	2 gm.
June 12	2,338.8	2,422.2	3.44	876.9	.6736	1.3686	2.85	daily
June 13	2,627.9	2,518.3	---	879.6	1,4877	2.0287	1.45	
	12,892.9	12,445.7	.68	3,122.7	7.513	9.62	14.13	
June 16	1,726.0	1,708.6	---	724.5	2.1105	2.8067	1.8409	
June 17	949.9	1,149.8	17.39	804.5	1.8126	2.5479	2.50	2 gm.
June 18	1,081.6	1,422.7	23.9	997.1	1.2889	2.3744	2.76	daily
June 19	828.6	1,084.4	23.58	962.6	.9487	1.9397	2.65	
June 20	1,112.5	1,371.5	18.89	968.7	2.015	2.704	2.45	
	5,698.6	7,737.0	16.7	4,457.4	8.158	12.34	12.20	
June 23	824.9	988.4	16.65	779.9	1.0081	1.3942	1.61	
June 24	1,019.3	1,320.4	22.80	1,044.7	1.4716	1.5430	2.36	
June 25	972.5	1,472.3	33.95	972.5	1.6951	2.3729	1.90	2 gm.
June 26	1,323.6	1,404.8	5.78	993.9	1.3832	2.2836	1.65	daily
June 27	975.1	1,152.5	15.40	724.1	1.2744	1.8923	1.71	
	5,115.4	6,337.4	18.9	4,515.1	6.818	9.47	9.23	
June 30	349.5	443.4	21.18	813.0	1.2948	2.5099	2.82	
July 1	435.9	558.3	21.93	845.4	2.3995	2.4378	4.18	no
July 2	392.4	503.3	22.24	822.3	2.1552	2.1552	3.41	hydro-
July 3	325.6	425.1	27.98	931.5	2.2356	2.7945	1.74	qui-
July 4	306.4	405.4	11.11	725.6	1.0125	1.5750	1.95	none
	1,809.8	2,331.5	21.08	4,137.8	9.07	11.44	14.10	

TABLE IV(Cont'd)

	Free phenol per day	Total phenol per day	% conjugation	Creatinine	Inorganic Sulf.	Total Sulf.	Total Nitrogen	
July 7	470.7	553.7	15.3	738.3	2.4479	2.6362	4.05	
July 8	485.1	627.2	22.67	803.5	1.5694	2.1014	3.27	no
July 9	351.1	512.07	31.42	872.3	2.4288	2.4288	3.89	hydro-
July 10	415.0	468.7	27.06	914.3	2.0230	2.0230	3.52	qui-
July 11	484.5	626.8	22.73	785.5	2.3985	2.3985	3.44	none
	2,206.4	2,788.47	23.8	4,113.9	10.83	11.561	18.12	
1st week	1,336.7	1,683.8	18.4	1,290.3	4.20	4.62	6.56)	no
2nd week	1,632.4	1,652.7	7.6	1,337.5	7.33	8.16	8.45)	hydro-
								qui-
								none
3rd week	5,448.2	5,419.3	1.7	2,441.8	6.33	7.65	---)	1 gm.
4th week	5,638.7	5,327.2	1.02	2,593.7	5.87	6.48	---)	daily
5 th week	6,740.7	6,292.5	.66	2,602.7	6.93	10.65	8.53	
6th week	2,336.5	2,245.8	---	2,818.03	7.48	10.47	3.33	no hydroquinone
7th week	12,892.9	12.445.7	.68	3,122.7	7.51	9.62	14.13)	2 gms.
8th week	5,698.6	7,737.0	16.7	4,457.4	8.15	12.34	12.2)	daily
9th week	5,115.4	6,337.4	18.9	4,515.1	6.81	9.47	9.23)	
10th week	1,809.8	2,331.5	21.08	4,137.8	9.07	11.44	14.10)	no
11th week	2,206.4	2,788.4	23.8	4,113.9	10.83	11.56	18.12)	hydro-
								qui-
								none

	1st.	2nd.	3rd.	4th.	5th.	6th.	7th.	8th.	9th.	10th.	11th.
% conjug. sulfates	9%	10%	17%	9%	35%	28%	21%	34%	28%	20%	6%

For the fifth experiment another animal was obtained. This animal weighed about 60 pounds, at the beginning of the experiment. The data are shown in Table V for free and total phenols, total sulfate and total nitrogen. It will be seen that these data are very similar to those of the preceding experiments. Upon administering hydroquinone there was an increase of both free and conjugated phenols, but little change in the percent of conjugation. About 49% of the hydroquinone was excreted as urinary "phenol". The total sulfate output was lowered slightly by the feeding of hydroquinone, the daily value of total sulfate averaging .38 gms. less than during the control period. This decrease in total sulfur elimination was more pronounced in the succeeding experiment (Table VI) but is also suggested by the data of experiment IV.

TABLE V

	Free Phenol	Total Phenol	% conjugation	Total Sul	Total Nitrogen	
Apr. 7	487.9	557.2	12.44	2.22	4.54	
Apr. 8	518.1	449.2	29.19	1.92	5.69	
Apr. 9	522.5	569.2	8.24	1.51	4.52	no hydro-
Apr. 10	586.5	693.02	15.36	2.19	5.71	quinone
Apr. 11	425.7	494.1	14.27	2.58	6.58	
Apr. 12	579.2	409.05	7.28	1.48	5.45	
Apr. 13	665.8	741.5	10.21	3.91	6.57	
Apr. 14	511.08	823.6	38.19	2.21	4.45	
	3,894.5	4,737.0	17.78	18.02	40,89	
Apr. 17	621.6	847.4	26.6	2.45	5.60	
Apr. 18	708.8	940.6	24.65	2.38	5.44	
Apr. 19	1,041.6	1,071.5	33.33	2.25	5.11	1 gm.
Apr. 20	1,134.0	1,193.9	5.02	2.54	6.25	hydro-
Apr. 21	1,083.2	1,137.4	4.74	1.91	5.04	quinone
Apr. 22	1,075.8	1,257.8	14.47	2.4	5.88	
Apr. 23	1,039.9	1,237.9	16.00	2.00	5.22	
Apr. 24	784.9	964.1	18.58	1.96	7.02	
	6,789.8	8,650.6	21.51	14.89	45.56	

The administration of hydroquinone seeming to have no serious effects on the experimental animals used, the author undertook a similar experiment using himself as the subject. In this experiment inorganic sulfate, total sulfate and total nitrogen determinations were determined, as given in Table VI. No attempt was made to control the diet during this experiment and this must be borne in mind in comparing the data with the previous experiments. Upon taking hydroquinone there was a very definite increase in conjugation of sulfates, about a 35% increase. The total sulfates showed a decrease which averaged about 1 gm. per day, while hydroquinone was being taken. Since the diet was not controlled the true significance of these values cannot be determined. There was an immediate definite increase in total nitrogen upon taking hydroquinone, the values soon returned to normal, however. The phenol determinations also were similar to those of of previous experiments, there being an increase in both free and conjugated phenols but no change in the percent of conjugation. About 65% of the hydroquinone appeared as urinary "phenol".

TABLE VI

	Free phenol	Total phenol	Ethereal phenol	% conjugation	Total N.	Inorganic Sulfate	Total Sulfate	% conjugation
Jan. 5	258.06	362.6	104.5	28.8	10.79			
Jan. 6	370.6	450.3	79.7	19.9	12.39			
Jan. 7	457.2	582.9	125.9	21.5	13.05			
Jan. 8	414.5	440.04	25.5	5.8	11.89			
Jan. 9	387.7	525.9	148.1	30.5	12.86			
	1,878.0	2,361.7	483.5	20.48	60.98			
Jan.12	1,039.2	1,147.5	108.3	9.4	17.11			
Jan.13	813.8	1,217.3	403.4	33.1	10.36			
Jan.14	871.9	1,304.2	433.3	33.1	11.10			
Jan.15	1,259.2	1,462.2	203.7	13.9	12.28			
Jan.16	1,062.4	1,148.6	86.1	7.5	9.90			
	5,046.5	6,279.8	1,234.8	19.64	60.75			
Jan.17	1,013.4	1,107.0	93.6	8.45	8.52			
Mar. 1						5.119	6.018	14.93
Mar. 2						4.436.	5.215	14.94
Mar. 3						5.885	6.828	13.97
Mar. 4						4.912	5.623	12.65
Mar. 5						4.140	4.727	12.43
Mar. 6						4.275	4.812	11.15
						28.567	33.223	14.03
Mar. 9						3.267	4.239	20.60
Mar. 10						2.683	3.570	24.87
Mar. 11						3.423	4.165	17.83
Mar. 12						4.165	5.528	24.69
Mar. 13						3.740	4.782	21.79
Mar. 14						3.810	4.700	18.94
						21.088	26.884	21.69

Isolation of Urinary Compounds

Attempts were made to isolate hydroquinone and muconic acid from the urine of animals receiving hydroquinone. For the isolation of hydroquinone the method of Baumann and Preusse (15) was used. The urine was heated after being made distinctly acid with HCl. It was then thoroughly extracted with ether. The ether extract was evaporated to dryness and the residue dissolved in water and neutralized with barium carbonate. This solution was then again extracted with ether until the water solution would no longer reduce Tollen's reagent in the cold. The ether extract was evaporated nearly to dryness and crystals of hydroquinone appeared. They were purified to some extent by recrystallization from toluene. However, the author was unable to entirely free the crystals from the pigment extracted with them.

In this manner crystals were obtained melting at 138° - 140°. The crystals sublimed without decomposition when heated with ferric chloride; dissolved in ammonia yielding a brownish liquid; sublimed, when heated rapidly in an open test tube, giving an indigo blue color, all of these properties corresponding to those of hydroquinone. Because the crystals could not be completely freed from pigment, their weight could not be accurately determined. Colorimetric analysis, however,

showed the ether extract of a two day urine sample of an animal receiving 1 gm. of hydroquinone daily to contain .0285 gms. of phenolic substance.

Several attempts were made to isolate muconic acid using the method of Neumaerker (3). The urine sample was evaporated to a syrup and extracted for twelve hours with ethyl acetate. The ethyl acetate was extracted by shaking with saturated sodium carbonate until no more carbon dioxide was evolved. The sodium carbonate solution was heated until the odor of ethyl acetate could no longer be detected. The solution was then neutralized with H Cl to congo blue. Muconic acid, if present, should have precipitated at this point. However, no muconic acid was found in four attempts.

The hydroquinone that was not excreted as a phenol, if oxidized, was probably carried beyond the stage of muconic acid.

Although there are many variations in the six experiments, due probably to differences in experimental animals, several facts stand out concerning the fate of hydroquinone in the animal body. In each case upon administering hydroquinone there was an immediate increase in both free and conjugated phenols in the urine. The percent of conjugation was changed but little, however, unless the amount of hydroquinone given was increased to at least 2 gms. daily. The percent of conjugation was then increased, the amount of the increase varying with individuals. The percent of the hydroquinone excreted as fed varied from 25% to 80%. At least a part of the hydroquinone was excreted in either the free or conjugated form without having been changed by passage through the body. The portion of hydroquinone unaccounted for by the urinary phenols may have been oxidized past the stage of muconic acid, presumably to carbon dioxide and water.

The total sulfate excretion was lowered by the feeding of hydroquinone. The decrease was most prominent in the last case (see table VI). The exact significance of this is open to discussion. It may be suggested, however, that Shiple, Muldoon and Sherwin (10) found total sulfates to be lowered when cystine was fed with C_6H_5 Br, due to the excretion of C_6H_5 Br. as a

mercapturic acid. The percentage of conjugation of sulfates was increased substantially in each case.

The excretion of total nitrogen and creatinine were both definitely stimulated upon administering hydroquinone. The values for both of these substances tended to regulate themselves toward the normal, however. This would suggest that hydroquinone causes an increase in the catabolism of body tissues, but that the animal body has a tendency to adjust itself to eliminate this extra tissue destruction.

These conclusions may be summarized briefly as follows:

The feeding of hydroquinone brings about,

1. Immediate increase in urinary phenols,
2. Little or no increase in the percent of conjugation of urinary phenols,
3. A slight decrease in total sulfate values,
4. A definite increase in ethereal sulfates,
5. A definite stimulation of creatinine and total nitrogen excretion, both of which tend to return to normalcy, however.

Hydroquinone, but no muconic acid, can be isolated from the urine of animals receiving 1 gm. of hydroquinone daily.

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