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Effects on Urinary Metabolites of Rats Fed Various Edible Fats

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EFFECTS ON URINARY METABOLITES OF RATS FED VARIOUS EDIBLE FATS

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INTRODUCTION

For many years, substitutes for butterfat have been the subject of much controversy. Their acceptance as being nutritionally equal to butterfat has often been questioned. The importance of this problem lies primarily in its economic and nutritional significance.

A recent study of public attitudes toward dairy products conducted by Alfred Politz Research, Inc. (39) for the American Dairy Association, shows that 48.4 per cent of the population believed that butter is a more nutritious food than oleomargarine. Only 6 per cent thought that oleomargarine is more nutritious while 27.2 per cent believed they are about the same. The remainder, 18.4 per cent, stated that they did not know.

Margarine tax laws were in force for some time while vegetable oils were being tested as a human food. In addition to chemical examination, many investigations were conducted with rats, calves, and humans. Although there were still conflicting results, the tax laws were repealed and substitutes went on the market to compete with butter. Many investigators still question the decision that substitutes are equal to butter, nutritionwise.

Many investigations (4, 6, 21, 34) have shown that butter contains one or more growth factors which have not, as yet been identified. Chemical methods have failed to identify any of these factors, and so other methods have

been considered. It is a well known fact that interrelationships exist between fat metabolism and the metabolism of other substances. It has been shown (11) that dietary fat affects the metabolism of other nutrients such as calcium and phosphorus. Although not clearly understood, it has been demonstrated (25, 37) that the kidney plays an important role in fat transport and metabolism. The value of urinalysis as a method of indicating the metabolism as a whole is well known, as well as is the high sensitivity of metabolic systems to minute quantities of some substances. The possibility of detecting growth factors or other substances through a study of excreted metabolites seems worthy of consideration in this study.

Recent work with paper chromatography at the University of Texas (48) has indicated that each individual has a specific urinary metabolic pattern. Similarities have been found in the patterns of identical twins and schizophrenics. It was also found that the diet changed this pattern to a certain extent, although this was not studied extensively.

The problem reported here is a study of urine obtained from highly inbred rats fed animal and vegetable fats analyzed by use of paper chromatography.

REVIEW OF LITERATURE

Chemical Differences

The main chemical differences between butterfat and vegetable oils are not so much in the type of compounds present but in their relative amounts. In general, butterfat (40) contains considerable quantities of all of the common fatty acids with myristic, palmitic, and oleic acids predominating. Vegetable oils (40) contain very small amounts of palmitic, oleic, and linoleic acids. Butterfat contains short chain fatty acids, vitamins, sterols, and other fat-like substances which are largely absent from vegetable oils.

Nutritional Differences

When vegetable oils first appeared on the market as a substitute for butter, they were rejected, generally, as inferior fats of low digestibility (14). This belief was supported by work of Deuel, et al. (15) and Bhalerao, et al. (2). In 1947, investigators in the Office of Home Economics of the U. S. Department of Agriculture (14) obtained digestibilities of approximately 95 per cent for all of the common vegetable oils. Today the high digestibility of margarine is generally accepted.

In 1913, it was found that butter was a good source of vitamin A which was absent from most vegetable oils (14). In 1929, Williams and Maclennan (51) recommended that

margarines be fortified with vitamins A and D. This was done in America as soon as the law permitted it. Fortified margarine now equals or exceeds average butter in vitamin A and D content (47). Deuel, et al. (23) found that the physiological availability of vitamin A in oleomargarine was 130 per cent, which seemed to be associated with the presence of added preservatives.

In 1941, Boer (4) announced the discovery of a new factor in the sebacic acids of butter which was necessary for the normal growth of rats. In 1947, he (5) isolated the so-called factor and found it to be vaccenic acid (11:12 octadecenoic acid). This discovery was made even more significant when Geyer and co-workers (30) claimed that no vaccenic acid could be found in the common vegetable oils. The nutritional significance of vaccenic acid stimulated further research by Boer, et al. (6), Deuel, et al. (20), Euler, et al. (29), Jansen, et al. (34), and Nath, (38). They all concluded that vaccenic acid plays no specific role in relation to growth of the rat.

One of the first claims against butter (47) came when it was discovered that cholesterol, the common sterol of butterfat, was related to atherosclerosis through the deposition of cholesterol-protein complexes in the walls of blood vessels. Studies (47) then showed that cholesterol is synthesized in the body independent of dietary intake.

Another important consideration when comparing fats

nutritionally, is their relative essential fatty acid content. Deuel and associates (24) have shown that margarines contained from 2.35 to 6.84 per cent while butter ranged from 1.1 to 4 per cent. They were both found to contain adequate amounts for most human needs.

Westerlund (50) reports that rats fed margarine soon developed negative calcium balances but retained more phosphorous than the rats fed butter. Work done by Dutta (26) and De, et al. (11) showed that hydrogenated vegetable fats and cocoanut oil were not equal to butter in maintaining calcium balances. Later, Deuel, et al. (22) reported that margarine gave slightly better calcification and slightly more positive calcium balances than butter in young rats. In adult rats there was no difference attributable to diet. The need for more information is evident.

Many investigators have made studies with rats on the over-all nutritive value of butter and margarine. Some of the first trials, in 1940 and 1941, at the University of Wisconsin under Schantz (42, 43, 44) and Boutwell (7) seemed to favor butter. These results were later minimized by Boutwell, et al. (8) when it was found that butter was only superior when the dietary carbohydrate was lactose.

Deuel and co-workers (16, 17, 18, 19, 22) concluded that fortified oleomargarine is nutritionally equal to butter when fed to rats with an otherwise adequate diet. This conclusion was also supported by work done by Barki, et al. (1), Bhalerao, et al. (3), Euler, et al. (27, 28),

and Smits (46). As a result, the committee on fats of the Food and Nutrition Board concluded (47):

> "The present available scientific evidence indicates that when fortified oleomargarine is used in place of butter as a source of fat in a mixed diet, no nutritional differences can be observed."

Similar studies with calves instead of rats have not always supported this conclusion. Work done by de Man (13), Gullickson (32), and Jacobson, et al. (33) found no vegetable oils other than hydrogenated soybean oil that were equal to butterfat when fed to calves.

A study by Leichanger, et al. (35) with children over a period of two years, concluded that margarine was equal to butter in promoting growth when used as a table fat.

METHOD OF PROCEDURE

Care of Rats

One hundred Sprague-Dawley, albino rats, weighing 42 to 58 grams were randomly assigned, other than sex, into 10 groups. Each group was assigned identical purified diets with the exception of type and/or amount of fat as shown in table 1. Butter oil, margarine oil, lard, soybean oil, and cottonseed oil were fed at 20 and 35 per cent levels of the total diet for 12 weeks. The butter and margarine oils were obtained by melting, centrifuging, and separating oil from curd by suction.

Table 1. Assignment of diets

Diet No.	Rat No.	Level and Type of Fat
1	1 through 10	20% butter oil
2	11 through 20	35% butter oil
3	21 through 30	20% margarine oil
4	31 through 40	35% margarine oil
5	41 through 50	20% lard
6	51 through 60	35% lard
7	61 through 70	20% soybean oil
8	71 through 80	35% soybean oil
9	81 through 90	20% cottonseed oil
10	91 through 100	35% cottonseed oil

A large amount of the basal diet (table 2) was mixed and stored to be used as needed. The fats and vitamins were added to the basal diet just prior to feeding. Vitamins as shown in table 3 were premixed with casein, and then fed at 0.9 per cent of the total diet at periodic intervals.

Table 2. Basal Diet

casein	27.8%
sucrose	66.7%
salts	5.5%

Salt Mixture

NaCl	27.99%
K_2HPO_4	26.32%
$Ca(H_2PO_4)_2 \cdot H_2O$	22.04%
$CaCO_3$	16.85%
$MgCO_3$	4.68%
$FeC_6H_5O_4 \cdot 3H_2O$	0.02%
$MnSO_4$	0.03%
$K_2Al_2(SO_4)_4 \cdot 24H_2O$	0.01%
KI	0.005%
$CaCl_2 \cdot 6H_2O$	0.01%
$ZnCO_3$	0.005%
$CuSO_4 \cdot 5H_2O$	0.02%

Table 3. Vitamin Mix

Thiamine (hydrochloride)	500 mg.
Riboflavin	600 mg.
Pyridoxine	300 mg.
Calcium Pantothenate	3 gm.
Niacin	6 gm.
Folic acid	100 mg.
Vitamin K	100 mg.
Vitamin B_{12}	0.5 mg.
Biotin	10 mg.
Inositol	15 mg.
(made up to 2,000 gm. with casein)	
(the following vitamins were added separately)	
Choline Chloride	0.1% of diet
Vitamin E	10 mg. / 100 gm. diet
Vitamin A concentrate	2000 I.U. / 100 gm. diet
Vitamin D	200 I.U. / 100 gm. diet

Each rat had diet and water available at all times during the 12 weeks of the experiment. Records were kept of body weights and feed consumption. The urine was collected in small bottles using a large plastic funnel under each cage. The urine samples were collected weekly and frozen until analyzed. No urine preservative was used.

Chromatographic study of urine

Paper chromatographic techniques have found wide application in analysis of biological fluids. Urography, or the paper chromatographic study of urine, was selected for this study because of the large number of urinary constituents that could be studied, its low cost, and simpleness. The ascending method, both one- and two-dimensional, was used. Methods developed by the University of Texas (48) were used as guides in developing the techniques.

Whatman No. 1 filter paper 9 X 11 inches was found to be most useful. Five samples of urine were run on each sheet with the one-dimensional method. Pencil marks were made an inch apart, one inch from the bottom of the paper to mark the spot where each sample was to be applied. Calibrated capillary pipettes were used to apply 0.01 ml. aliquots of sample on the pencil marks. Four aliquots were placed on the same spot allowing each one to dry before applying the next. The use of this procedure kept the area of the spot as small as possible and still allowed for the application of sufficient volume of urine to insure a good chromatogram.

When the spots were completely dry the paper was stapled in the form of a cylinder with the spots at the bottom. Acid resistant paper clips instead of staples were used with acidic solvents. Twenty ml. of freshly prepared solvent was added to a vinyl plastic dish, six inches in diameter, which was placed on the bottom of a wide-mouthed

gallon jar. The cylindrical chromatogram was placed upright in the solvent with the spots at the bottom to allow ascension of urinary constituents with the solvent. A suitable cap, which increased the length of the jar enough for the chromatogram (see plate 1), was made from a cottage cheese carton. After the cap was placed on the jar, the cylinder was left until the solvent reached the top of the paper. The time required for ascension of the solvent varied from 9 to 16 hours, depending on the type of solvent and its water content.

After the solvent had ascended to the top of the sheet, the cylinder was removed and allowed to dry at room temperature. The staples were then removed and a color reagent employed to bring out the spots. Color reagents were applied to the sheet by spraying or dipping. Ninhydrin was the color reagent used most generally with all techniques. Ultra-violet light was used before and after color development. All of the spots were outlined to prevent loss of identity by fading.

Two-dimensional chromatograms were run in a similar manner. One sample was placed in a corner of the sheet, one inch from the edges. Six 0.01 ml. aliquots of urine were used to produce more clearly delineated spots. The first solvent was run lengthwise and the cylinder was restapled so that the second solvent could be run widthwise. It was necessary to completely dry the sheet after the use of the first solvent or the spots would smear and leave

Plate 1. Paper chromatography apparatus

streaks. The spots were systematically recorded and examined for differences associated with dietary fat.

RESULTS AND DISCUSSION

Rats

The average growth rates of rats fed two levels of five different fats are shown graphically in figures 1 and 2. More rapid growth rates with less variation occured with the 20 per cent fat diets. A total of 16 rats died during the 12 weeks of the experiment. Of the 20 rats that were fed each fat, two rats died that were fed butter, three rats died that were fed margarine, six on lard, two on soybean oil, and three on cottonseed oil. The time at which these deaths occured is noted by a (▲) on the growth rate curve of each diet. The causes of death were not determined.

As near as could be determined, the healthier rats were found on the diets with the more rapid growth rates. There were a few visible signs of health disturbances but none that were consistant within a group fed a particular fat diet. The smaller rats on the 35 per cent vegetable oil diets, especially cottonseed, were often covered with oil.

The average total food consumption for the 10 rations is shown in figure 3. Rats that died during the experiment were not included. In each case the rats on the 35 per cent fat diets were unable to consume as much as those on the 20 per cent diets.

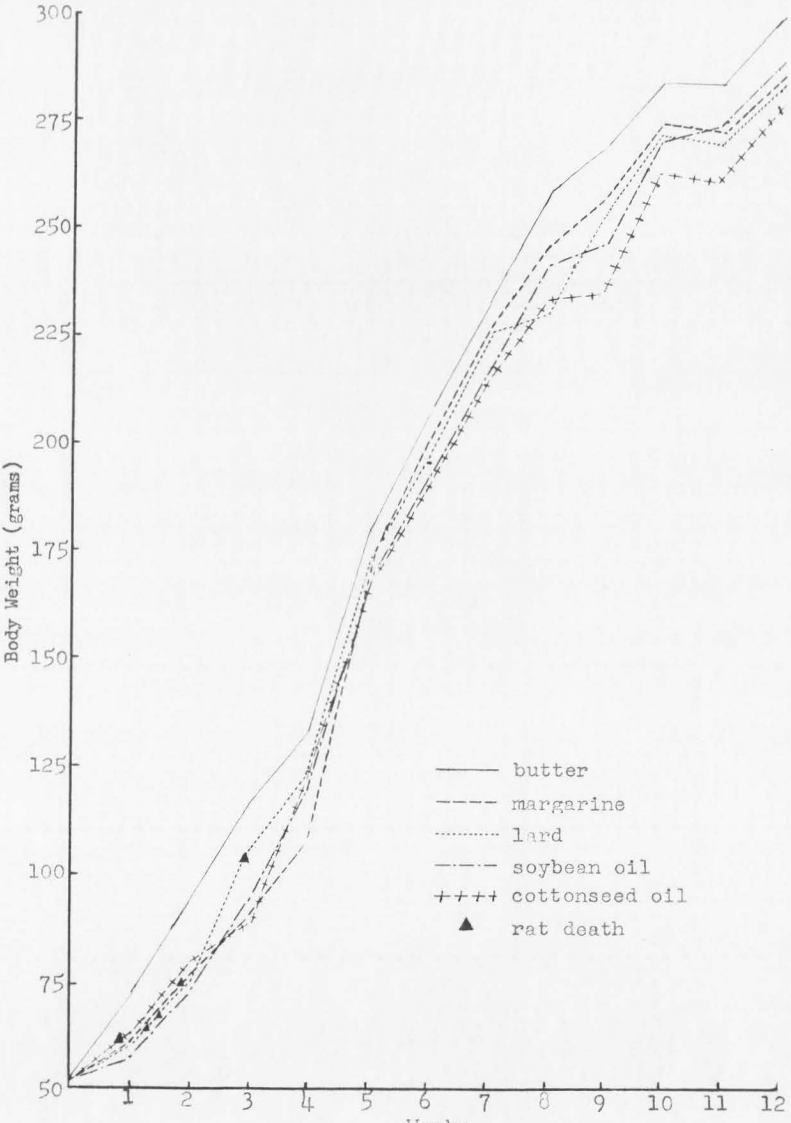

Figure 1. Average rat growth rates on 20% fat diets

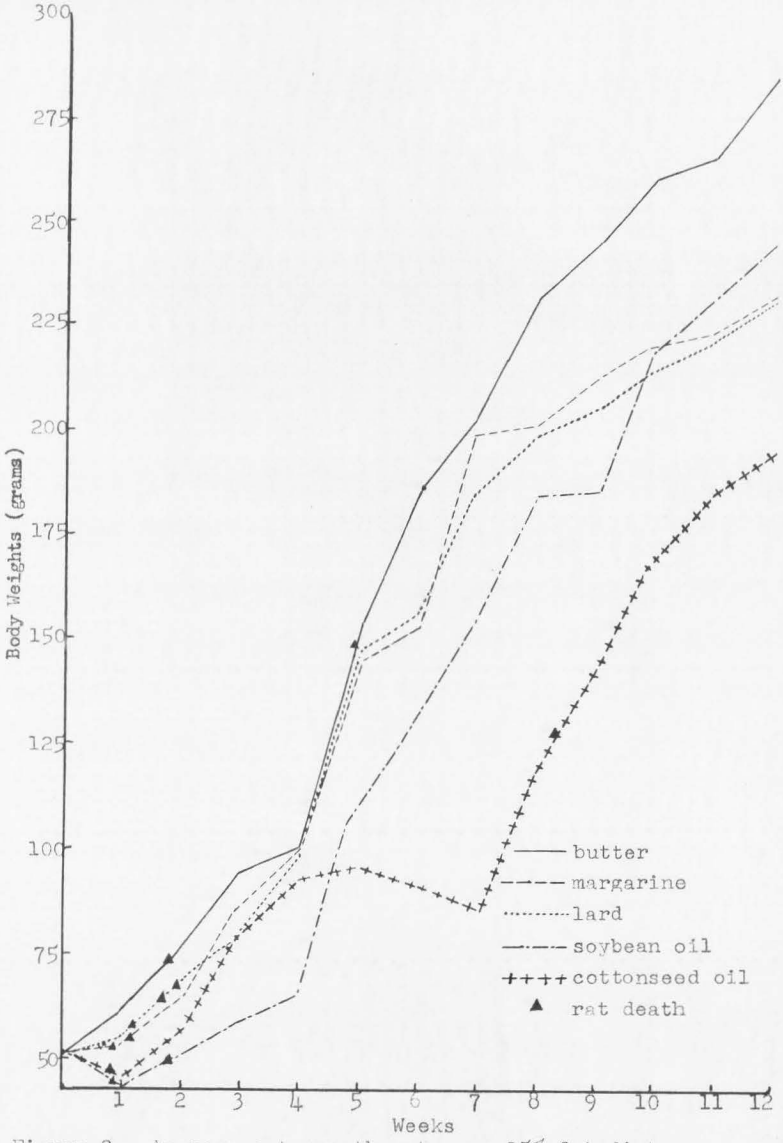

Figure 2. Average rat growth rates on 35% fat diets

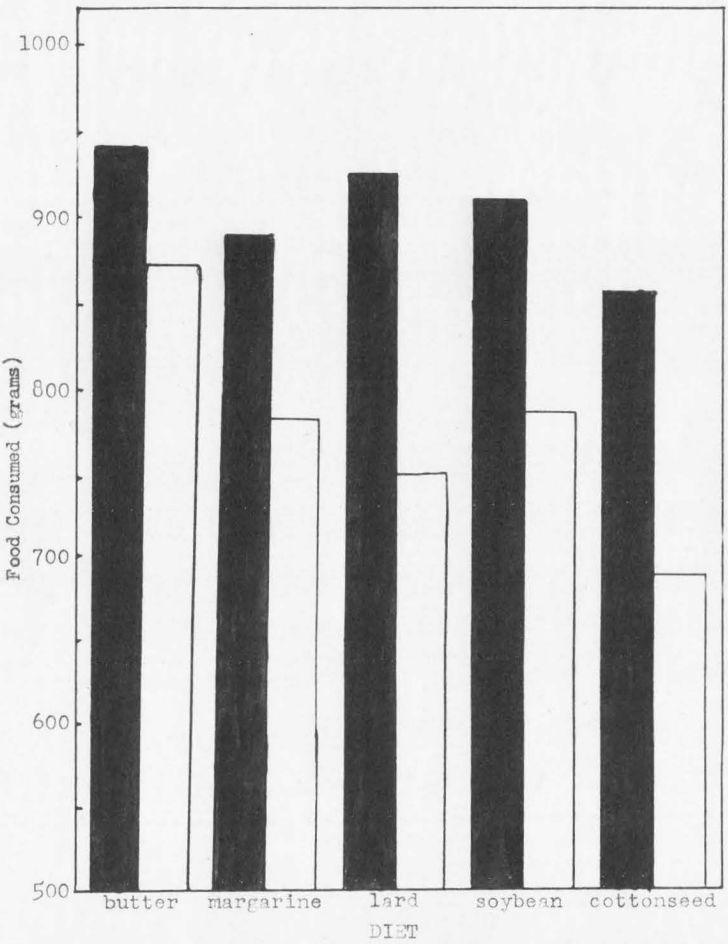

Figure 3. Average total food consumed by rats fed diets containing five different types of fat, each at 20 (white bars) and 35% (black bars) of the diet

Chromatography

Phenol, one-dimensional, urograms were the first to be run. The 1st, 8th, 9th, 10th, and 11th week samples for each rat were run on the same sheet for the sake of comparison. After being colored with ninhydrin, the prominant spots were outlined under the ultra-violet light. The frequently occurring spots were designated by the numbers one through eight. Each of these spots is described in table 4. The distance that a spot is carried divided by the distance traveled by the solvent is known as the Rf. value. Since a large variation in Rf. values was obtained, a range or average value is given in the tables.

Table 4. Description of spots found on phenol, one-dimensional urograms (see plate 2)

Spot No.	Rf. Range	Color	Characteristics
1	.85 - .90	bright blue(Nin.)	irregular, fades slow
2	.75 - .90	purple(Nin.)	irregular, indistinct
3	.65 - .80	light(U.V.)	round, indistinct
4	.45 - .65	purple(Nin.)	large, distinct
5	.35 - .45	light(U.V.)	round, not distinct
6	.25 - .40	purple(Nin.)	indistinct
7	.20 - .25	bright blue(U.V.)	thin, curved
8	.05 - .20	purple(Nin.)	elongated

The spots are recorded as numbered in table 4 in tables 5 through 9 for the first series.

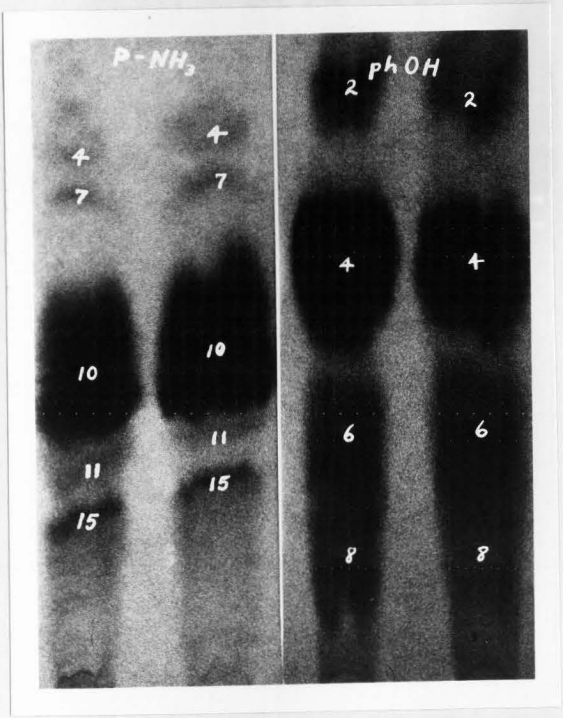

Plate 2. Examples of phenol and propanol-ammonia, one-dimensional urograms with identifying numbers on visible spots

Table 5. Presence (*) or absence (-) of spots on phenol, one-dimensional, urograms from 1st, 8th, 9th, 10th, and 11th week samples from rats fed diets containing butter oil*

Rat No.	Spot Number 1	2	3	4	5	6	7	8
1	-----	*****	-----	*****	-----	*****	-****	*****
2	-----	*****	-----	*****	-----	*****	****	*****
3	-****	*****	-----	*****	-----	*****	-****	*****
4	-****	*****	-----	*****	-----	*****	****	*****
5	-****	*****	-----	*****	-----	*****	-****	*****
6	*****	*****	-----	*****	-----	*****	-****	*****
7	*****	*****	-----	*****	-----	*****	-****	*****
8	-****	*****	--***	*****	-----	*****	-****	*****
9	*****	*****	-----	*****	-----	*****	-****	*****
10	*****	*****	-****	*****	-----	*****	-****	*****
11	*****	*****	-----	*****	-----	*****	-****	*****
13	*--**	*****	--*--	*****	-----	*****	-****	*****
14	----	****	----	****	----	****	****	****
15	-----	*****	-----	*****	-----	*****	-****	*****
16	*****	*****	-----	*****	-----	*****	-****	*****
17	-****	*****	-----	*****	-----	*****	-****	*****
18	*****	*****	--***	*****	-----	*****	-****	*****
20	--***	*****	--*--	*****	-----	*****	-****	*****

* The five values for each spot number represent the 1st, 8th, 9th, 10th, and 11th week samples, respectively.

Table 6. Presence (*) or absence (-) of spots on phenol, one-dimensional, urograms from 1st, 8th, 9th, 10th, and 11th week samples from rats fed diets containing margarine oil*

Rat No.	Spot Number 1	2	3	4	5	6	7	8
21	-*****	*****	--**-	*****	-----	*****	-****	*****
22	*****	*****	--****	*****	-----	*****	-****	*****
23	-*****	*****	--**-	*****	-----	*****	-****	*****
24	-*****	*****	-----	*****	-----	*****	-****	*****
26	*****	*****	--****	*****	-----	*****	-****	*****
27	-*****	*****	-****	*****	-----	*****	-****	*****
28	-*****	*****	-----	*****	-****	*****	-****	*****
29	-*****	*****	-----	*****	-----	*****	-****	*****
30	-*****	*****	--****	*****	-----	*****	-****	*****
31	-----	*****	-----	*****	*****	*****	*****	*****
32	-----	*****	-----	*****	---**	*****	*****	*****
34	--****	*****	-----	*****	---*-	*****	*****	*****
35	-*****	*****	-----	*****	-****	*****	*****	*****
36	-----	*****	---*-	*****	-****	*****	-****	*****
38	-*****	*****	--****	*****	--****	*****	*****	*****
39	-*****	*****	-----	*****	-----	*****	-****	*****
40	*****	*****	----	****	*-****	*****	****	****

* The five values for each spot number represent the 1st, 8th, 9th, 10th, and 11th week samples, respectively.

Table 7. Presence (*) or absence (-) of spots on phenol one-dimensional, urograms from 1st, 8th, 9th, 10th, and 11th week samples from rats fed diets containing lard*

Rat No.	Spot Number 1	2	3	4	5	6	7	8
42	*****	*****	-*-*-	*****	--*--	*****	*****	*****
44	-----	-----	-----	*****	****-	*****	*****	*****
45	-----	--*--	--*--	*****	*****	*****	*****	*****
46	-----	**---	-----	*****	-****	*****	*****	*****
47	-----	**---	-----	*****	-****	*****	*****	*****
48	*****	*****	---*-	*****	--***	*****	*****	*****
50	-----	*****	*----	*****	-----	*****	*----	*----
51	-****	*****	-----	*****	-****	*****	*****	*****
55	*****	*****	--*-	*****	*****	*****	*****	*****
56	-****	*****	--***	*****	-****	*****	*****	*****
57	*****	*****	-----	*****	*****	*****	*****	*****
58	-----	*****	-----	*****	*****	*****	*****	*****
59	*****	*****	-----	*****	--*--	*****	****-	*****
60	-----	*****	-----	*****	-***-	*****	*****	*****

* The five values for each spot number represent the 1st, 8th, 9th, 10th, and 11th week samples, respectively.

Table 8. Presence (*) or absence (-) of spots on phenol one-dimensional, urograms from 1st, 8th, 9th, 10th, and 11th week samples from rats fed diets containing soybean oil*

Rat No.	Spot Number 1	2	3	4	5	6	7	8
61	-----	*****	-----	*****	-----	*****	*****	*****
62	-****	*****	-----	*****	--**-	*****	-****	*****
63	-****	*****	-----	*****	-----	*****	*****	*****
64	-----	*****	-----	*****	--**-	*****	****-	*****
65	*----	*****	-----	*****	*----	*****	*---*	*****
66	*****	*****	--**-	*****	-----	*****	-****	*****
67	****-	*****	--**-	*****	-----	*****	*****	*****
68	*****	*****	-----	*****	-----	*****	****-	*****
69	-****-	*****	--**-	*****	-----	*****	*****	*****
70	*****	*****	-----	*****	-----	*****	*****	*****
71	-----	*****	-----	*****	--***	*****	*****	*****
72	-****	*****	-----	*****	-----	*****	*****	*****
73	*****	*****	--***	*****	-----	*****	-****	*****
74	-****	*****	--**-	*****	--***	*****	**** *	*****
76	-----	*****	--***	*****	-****	*****	*****	*****
77	-****-	*****	-----	*****	----*	*****	****-	*****
78	---**	*****	---**	*****	---**	*****	-****	*****
79	--***	*****	--**-	*****	---*-	*****	-****	*****

* The five values for each spot number represent the 1st, 8th, 9th, 10th, and 11th week samples, respectively.

Table 9. Presence (*) or absence (-) of spots on phenol, one-dimensional, urograms from 1st, 8th, 9th, 10th, and 11th week samples from rats fed diets containing cottonseed oil*

Rat No.	Spot Number 1	2	3	4	5	6	7	8
81	-----	*****	-----	*****	--***	*****	*****	*****
82	-----	*****	-----	*****	-----	*****	*****	*****
84	-****	*****	---*-	*****	-----	*****	-****	*****
85	-----	*****	--*--	*****	--***	*****	*****	*****
86	-****	*****	-----	*****	-----	*****	-***-	*****
87	--***	*****	-----	*****	-----	*****	-****	*****
88	--***	*****	-----	*****	-----	*****	-****	*****
89	-****	*****	-----	*****	-----	*****	-****	*****
90	-----	*****	--*--	*****	-----	*****	-****	*****
92	-****	****-	-----	*****	-----	****-	-***-	*****
93	*****	*****	-----	*****	-----	*****	-****	*****
94	-----	*****	-----	*****	-----	*****	-**--	*****
95	-----	*****	-----	*****	-----	*****	-----	*****
96	-----	*****	-----	*****	-----	*****	--***	*****
98	-****	*****	-----	*****	-----	*****	-****	*****
99	-----	*****	-----	*****	-----	*****	-***-	*****
100	-----	*****	-----	*****	-----	*****	-***-	*****

* The five values for each spot number represent the 1st, 8th, 9th, 10th, and 11th week samples, respectively.

A second series was run on the same samples as before with propanol-ammonia as the solvent. They were examined under the ultra-violet light both before and after treatment with ninhydrin. Twenty spots were examined and recorded in tables 11 through 15 in the same manner as the first series. A description of these spots appears in table 10.

Table 10. Description of spots found on propanol-ammonia, one-dimensional urograms. (see plate 2)

Spot No.	Rf. Range	Color	Characteristics
1	.85 - .98	yellow(U.V.)	small, round
2	.80 - .90	dingy, light(U.V.)	round, not distinct
3	.80 - .85	pink(Nin.)	extends from No. 4
4	.75 - .85	purple(Nin.)	associated with No. 3
5	.70 - .75	green(U.V.)	thin, blends with No. 6
6	.65 - .70	blue(U.V.)	thin, blends with No. 5
7	.60 - .70	purple(Nin.)	small
8	.50 - .60	dingy, light(U.V.)	round, not distinct
9	.55 - .65	red(U.V.) purple (Nin.)	curved around No. 9
10	.30 - .55	purple(Nin.)	large
11	.25 - .35	purple(Nin.)	associated with No. 12
12	.25 - .35	brown(Nin.)	associated with No. 11
13	.25 - .35	yellow(Nin.)	visible only when not covered by 11 or 12
14	.20 - .25	purple(Nin.)	thin
15	.20 - .25	purple(Nin.)	often covers 14 and 16
16	.20 - .25	green(U.V.)	thin, often hidden
17	.15 - .20	red(U.V.)	not distinct
18	.10 - .15	blue(U.V.)	thin
19	.05 - .15	blue(U.V.)	round
20	.05 - .10	purple(Nin.)	not distinct

Table 11. Presence (*) or absence (-) of spots on propanol-ammonia, one-dimensional urograms from 1st, 8th, 9th, 10th, and 11th week samples from rats fed diets containing butteroil*

Rat No.	Spot Number 1	2	3	4	5	6	7	8	9	10
1	-----	-*****	*----	*----	*****	-----	*----	****-	-----	*****
2	*****	-*****	*----	**---	*****	-----	**---	*-*--	-----	*****
3	-*****	-*****	-----	*****	-*****	-----	*****	-*****	-----	*****
4	*****	*****-	----*	----*	*****	-----	----*	*****	-***-	*****
5	*****	*****	*----	----*	*****	*****	-----	-*****	-----	*****
6	****-	****-	**---	**---	*****	-----	*----	****-	-----	*****
7	-*****	*****	*----	*----	*****	*****	*----	-----	-----	*****
8	****-	*****	**---	*----	*****	*****	----*	*****	-----	*****
9	--***	*****	*----	**---	*****	*****	**---	*****	-----	*****
10	-*****	*****	*----	**---	*****	-----	*----	---**	-----	*****
12	-*****	*****	*****	-----	*****	*****	-----	-----	*****	*****
13	-***-	*****	-*---	-*---	*****	-***-	-*---	*****	-----	*****
14	-*****	*****	**---	**---	*****	-*-**	-----	*****	-----	*****
15	*****	*****	**---	**---	*****	*****	**---	*****	--****	*****
16	****-	*****	**---	*----	*****	*****	*----	*****	*****	*****
17	-*****	*****	**---	*----	*****	*****	-----	**---	*****	*****
19	-*-**	*****	**---	**---	*****	*****	**---	*****	-----	*****
20	-****-	*****	**---	**---	*****	-*****	**---	*****	-----	*****

(continued)

Rat No.	Spot Number 11	12	13	14	15	16	17	18	19	20
1	*****	-***-	-----	*****	*****	-----	-----	-----	*****	*----
2	****-	-***-	-***-	*****	****-	*****	-----	*****	-----	*----
3	*****	-*****	-----	-*****	*****	-----	-----	-----	*****	-----
4	-----	--**-	-***-	*****	****-	*****	-----	****-	-----	-----
5	*****	****-	-----	*****	*****	****-	-----	*****	-----	-----
6	-----	-----	-----	*****	*****	-*****	-----	*****	-----	-----
7	*****	-***-	-*****	*****	*****	*****	-----	*****	-----	-----
8	*----	-*****	-*****	*****	*****	*****	-----	-----	*****	-----
9	*----	-*****	-***-	*****	*****	*****	-----	-----	*****	*----
10	-***	*****	----	*****	-***	*****	-----	----	*****	----
12	*****	-*****	-----	*****	*****	*****	-----	-----	*****	*----
13	-----	-***-	----*	*****	*****	----*	-----	*----	-*****	*----
14	*****	-***-	-----	*****	*****	*****	-*****	---*-	-----	-----
15	*****	-*****	-----	*****	*****	*****	-----	*****	-****	*----
16	*****	-*****	-----	*****	*****	*****	-----	-----	*****	*----
17	*****	-***-	-----	*****	*****	-*****	-----	*****	-*****	*----
19	*****	-*-**	--**-	*****	*****	*****	-----	*****	*****	*----
20	*****	-*****	-----	*****	*****	*****	-----	*****	-*****	*----

* The five values for each spot number represent the 1st, 8th, 9th, 10th, and 11th week samples, respectively.

Table 12. Presence (-) or absence (-) of spots on propanol-ammonia, one-dimensional urograms from the 1st, 8th, 9th, 10th, and 11th week samples from rats fed diets containing margarine oil*

Rat No.	Spot Number 1	2	3	4	5	6	7	8	9	10
21	-*****	*****	**---	*****	*****	*****	*****	*****	-----	*****
22	-*****	*****	**---	*****	*****	-***-	**---	*****	-----	*****
23	--***-	*****	-----	*****	*****	****-	**---	*****	-----	*****
24	*-***-	*****	**---	*****	*****	*****	*****	*****	-----	*****
26	*****	*****	-----	**---	*****	--***	-***-	*****	**---	*****
27	*****	*****	-----	**---	*****	*****	**--*	****-*	-----	*****
28	-**--	-***-	*----	**---	***--	****-	**---	*****	-***-	*****
29	-***-	*****	-----	***--	-***-	*****	***--	*****	-----	*****
30	-*****	-***-	-----	*****	-***-	-***-	*-***-	-*****	-----	*****
31	-*****	*****	-----	*****	-----	*****	**-**	*****	-----	*****
32	*****-	*****	*----	**---	*****	-----	*----	-***-	-***-	*****
34	*****	*--**	**---	**---	--***	*****	*****	-----	*-***-	*****
35	*----	-----	-----	*----	*****	-----	*----	*****	*****	*****
36	**--*	*****	**---	**---	*****	-***-	**---	*****	-----	*****
38	-----	*****	-----	*----	--***	****-	*----	*****	*****	*****
39	-*****	*****	-----	**---	-***-	-***-	**---	*****	-*****	*****
40	-***-	*****	**--	**--	*****	*****	**--	*****	-***-	*****

(continued)

Rat No.	Spot Number 11	12	13	14	15	16	17	18	19	20
21	*****	-***-	-----	*****	*****	*****	-----	-----	*****-	*----
22	*****	-***-	-----	*****	*****	-***-	-----	-----	-*****	*----
23	*****	-***-	-----	*****	*****	*****	-----	-----	*****	*----
24	*****	-***-	-----	*****	*****	****-	-----	-----	*****	*----
26	*****	-***-	-----	*****	*****	*--**	-----	*****	*****	-----
27	*****	-***-	-----	*****	*****	****-	-----	*****	*****	*----
28	*----	--*--	-***-	*****	****-	*****	----*	*****	-----	-----
29	*----	-**--	-***-	*****	*****	*****	-----	*----	-***-	*----
30	*----	-***-	-***-	-***-	****-	-***-	-----	-----	-***-	-----
31	*----	-----	*****	-----	****-	*****	-----	-----	*----	*****
32	*----	--***-	-***-	*****	*****	*****	-***-	-----	-----	-----
34	**---	---**	--***	--***	*****	*****	-----	*****	-***-	*----
35	-----	-----	---**	*****	*****	*****	-----	*****	*----	-----
36	*****	-***-	-***-	*****	*****	*****	----*	-----	-***-	-----
38	*-***-	--**-	-*****	-----	*****	*****	-----	*****	-----	*----
39	*----	-***-	-***-	*****	*****	*****	-----	-----	-----	-----
40	*----	-***-	--**	****	*****	-***-	-***-	----	*****	----

* The five values for each spot number represent the 1st, 8th, 9th, 10th, and 11th week samples, respectively.

Table 13. Presence (*) or absence (-) of spots on propanol-ammonia, one-dimensional, urograms from the 1st, 8th, 9th, 10th, and 11th week samples from rats fed diets containing lard*

Rat No.	Spot Number 1	2	3	4	5	6	7	8	9	10
42	-***-	*****	*----	*--**	*****	*****	**--*	-----	*-*-*	*****
44	*****	*****	-----	**--*	*****	*****	**---	*****	-----	*****
45	*****	*****	-----	**---	*****	*****	**---	*****	-----	*****
46	-**--	--***	*----	**---	*****	*****	**---	-***-	-----	*****
47	--*--	*****	*----	**---	-----	*****	*****	**-**	----*	*****
49	*****	*****	*----	**--*	--***	*****	*****	***-*	**---	*****
50	--*--	***--	*----	***--	*****	*****	***--	***--	-**--	*****
51	-----	*****	*----	*----	--***	*****	*----	*****	-****	*****
55	-**--	-****	-----	-----	*****	*****	-----	-----	*****	*****
56	---**	*****	-----	**---	-****	*****	**---	-****	-----	*****
57	-**--	*****	***--	***--	----*	*****	**---	--**-	--**-	*****
58	***--	*****	-----	**---	-****	*****	**-*-	*****	-----	*****
59	*----	*****	-----	-----	*****	*****	-----	*****	*----	*****
60	--*--	*****	-----	**---	*****	-****	-****	**---	-****	*****

(continued)

Rat No.	Spot Number 11	12	13	14	15	16	17	18	19	20
42	*----	-***-	----*	-***-	*-***	*****	-----	-----	*****	-----
44	*-**-	--**-	-*-**	-***-	*****	*****	-----	*---*	-***-	-----
45	-----	-***-	--***	*****	****-	-****	-----	*----	-***-	-----
46	-----	-***-	-****	*****	-****	*****	-----	*****	*****	*----
47	*----	-***-	-****	*****	***-*	*****	**--*	-----	-**-*	*----
49	*----	-***-	-****	*****	***--	*****	-----	-----	*****	*----
50	*----	--*--	-****	-***-	****-	-**--	-----	-***-	*----	*----
51	*----	-***-	-****	*****	*****	-****	-----	*****	-----	*----
55	*----	-***-	--***	*****	*****	*****	-----	*****-	----*	*----
56	*****	-***-	----*	*****	-****	*****	-----	*****	-----	*----
57	*****	-***-	----*	*****	-****	*****	-****	-----	-***-	*----
58	*----	-***-	----*	*****	-****	-****	-----	-----	-----	-----
59	*-**-	--**-	-----	-***-	*****	*****	-----	*****	-----	-----
60	*****	-***-	-****	-***-	*****	*****	-----	-----	**---	-----

* The five values for each spot number represent the 1st, 8th, 9th, 10th, and 11th week samples, respectively.

Table 14. Presence (*) or absence (-) of spots on propanol-ammonia, one-dimensional, urograms from the 1st, 8th, 9th, 10th, and 11th week samples from rats fed diets containing soybean oil*

Rat No.	Spot Number 1	2	3	4	5	6	7	8	9	10
61	*****	*****	-----	*---*	*****	---**	--***	-****	-----	*****
62	-*---	-*****	-*---	*****	*****	-***-	*****	-*****	-----	*****
63	****-	*****	-----	*****	*****	****-	*****	*****	-***-	*****
64	***--	*****	-----	*****	*****	-**--	*****	----*	-----	*****
65	***--	*****	-----	*****	*****	**---	*****	*****	-----	*****
66	*****	-*****	-----	*****	*****	-****	*****	-*****	***--	*****
67	***--	*****	**---	*****	*****	*****	*****	*****	*----	*****
68	--**-	--**-	-----	**---	*****	--****	*----	*-***	*-***	*****
69	-*****	*****	**---	*****	*****	*****	*****	*****	---**	*****
70	*****	*****	-----	**--*	-*****	*****	*****	*****	-----	*****
71	-----	-*****	-----	**---	-*****	-*****	**---	-*****	-----	*****
72	-----	*****	*---*	***-*	*****	*****	*---*	*****	-***-	*****
73	-----	*****	-----	*****	-*****	-*****	*****	*****	-----	*****
74	-----	***-*	*----	*****	*****	*****	*****	--***	-----	*****
76	-----	*****	*----	**---	*****	*****	*-**-	*****	-*---	*****
77	-----	*****	*----	*****	*****	*****	*****	*****	-***-	*****
78	-----	*****	*----	*****	*****	*****	**-**	-*****	-----	*****
79	-----	-*****	-----	**---	*****	*****	*****	*****	-----	*****

(continued)

Rat No.	Spot Number 11	12	13	14	15	16	17	18	19	20
61	*----	-*****	-*****	-*****	*****	*****	-----	-----	-----	-----
62	*----	-***-	-*****	-----	*****	*****	-----	-----	-----	-----
63	*----	-***-	-*****	-*****	*****	*****	-----	----*	-----	-----
64	*----	-**--	-*****	-***-	*****	***--	-----	--*****	-----	-----
65	*---*	-----	-*****	*****	*****	***--	-----	**---	-----	*----
66	*----	-*****	--*****	*-*--	*****	*****	-----	-*****	-----	-----
67	**---	-***-	--*****	-**--	*****	*****	-----	*****	-----	-----
68	*---*	--***-	--**-	*****	-*****	*****	-----	--*****	-----	*----
69	*----	-***-	--*-*	***--	*****	-***-	-----	*****	-----	*----
70	*----	-*****	-*****	*****	*****	*****	-----	-*****	-----	*----
71	*----	-**-*	----*	-*****	*****	-*****	*****	*****	-----	-----
72	*--*-	-***-	-----	*****	*****	*****	-----	***--	-----	*----
73	*----	-*---	-----	-*****	*****	*****	-----	*----	--**-	-----
74	****-	-***-	----*	*****	*****	*****	-----	-----	-----	*----
76	*----	--**-	-*****	*****	*****	*****	-----	*****	-----	*----
77	****-	-***-	-*****	*****	*****	*****	-----	*****	-----	*----
78	****-	-*****	-*-**	*****	*****	*****	-----	**-**	-----	-----
79	*----	-*****	----*	*****	*****	*****	-----	-*****	-----	-----

* The five values for each spot number represent the 1st, 8th, 9th, 10th, and 11th week samples, respectively.

Table 15. Presence (*) or absence (-) of spots on propanol-ammonia, one-dimensional, urograms from the 1st, 8th, 9th, 10th, and 11th week samples from rats fed diets containing cottonseed oil*

Rat No.	Spot Number 1	2	3	4	5	6	7	8	9	10
81	-----	*****	-----	**---	-****	-----	*****	*****	-----	*****
82	-----	*-***	**---	****-	*****	-****	****-	*****	*----	*****
84	-----	-****	-**-*	*****	-****	-****	*****	-****	-----	*****
85	-----	*****	**---	*****	*****	-**-*	*****	-****	-----	*****
86	-----	*****	**---	****--	*****	-***-	****-	*-****	**---	*****
87	-****-	*****	**--*	****--	-****	-****	****-	-****	-----	*****
88	--**-	-----	*----	*-***	--***	*-***	*-***	*****	-----	*****
89	*****	-----	*----	****--	*-*--	*****	****-	*****	-----	*****
90	-****-	-----	**---	*****	*****	*****	**--*	*****	-----	*****
92	-****-	-----	**---	**----	*****	*****	**---	*****-	----*	*****
93	-*****	-----	-----	**----	-****	*****	**---	*****	*****	*****
94	--**-	---**	-----	**---	-*---	-**--	*----	--**-	-----	*****
95	----*	*----	-----	**-**	---**	**-**	**---	**--*	-----	*****
96	---**	---**	*----	***-*-	---**	**-**	**---	*----	-----	*****
98	***--	-----	-----	*****	*****	*****	****-	-*****	-----	*****
99	---*-	-----	-----	-----	-****	-----	-----	*****	-----	*****
100	---*-	-----	-----	-***-	---**	-****	-----	-****	-----	*****

(continued)

Rat No.	Spot Number 11	12	13	14	15	16	17	18	19	20
81	*----	-***-	-*****	*****	*****	-*---	-----	-----	-----	-----
82	*---*	-***-	---*-	--***	*****	*****	-----	*-*--	-----	-----
84	-****	-*****	-----	-***-	*****	-*---	-----	*---*	-----	-----
85	*----	-*****	-*****	****-	*****	*****	-----	--****	-----	-----
86	-----	--**-	--***	*****	*****	*-***	-----	-**-*	-----	*----
87	**---	--**-	---*-	*****	*****	*****	-----	*-**-	-----	**---
88	*----	--**-	--***	*-***	*****	*-***	--**-	*----	-----	*----
89	***--	-*****	--***	*****	*****	*****	-----	-****	-----	*----
90	**---	--**-	--**-	*****	*****	-****	-----	-****	-----	*----
92	*****	-***-	--*--	*****	*****	*****	-----	*****	-----	*----
93	*----	-**-*	---**	*****	*****	*****	-----	*----	-----	-----
94	*-*--	--*--	--*-*	**--*	*****	*****-	--****	--****	-----	-----
95	*****	-----	-----	**-**	*****	**-**	-----	-----	-----	-----
96	*-*--	---**	-----	*****	*****	*****	-----	*--**	-----	*----
98	***--	-----	-----	-*****	*****	*****	*****	*---*	-----	*----
99	***--	-***-	----*	*****	*****	*****	-----	*****	-----	*----
100	*****	-***-	-----	*****	*****	*****	-----	*****	----+	-----

* The five values for each spot number represent the 1st, 8th, 9th, 10th, and 11th week samples, respectively.

The third series (phenol-lutidine, two-dimensional) was run on the 11th week samples first. The results are recorded in Tables 17 through 21 in the same manner as before except the Rf. values for lutidine were measured to the phenol front. The 20 spots examined are described in Table 16.

Table 16. Description of spots found on phenol-lutidine, two-dimensional urograms (see plate 3)

Spot No.	Ave. Rf. values Phenol	Lutidine	Color	Characteristics
1	.80	.70	red(U.V.) brown(Nin.)	distinct
2	.75	.50	blue(U.V.)	covered by No. 9
3	.70	.65	green(U.V.)	covered by No. 11
4	.70	.95	blue(U.V.)	thin
5	.55	.85	blue(U.V.)	small
6	.40	1.10	blue(U.V.)	beyond phenol front
7	.25	.40	blue(U.V.)	small
8	.25	.50	yellow(U.V.)	small, round
9	.75	.50	purple(Nin.)	covers No. 3
10	.65	.65	purple(Nin.)	just below No. 1
11	.75	.80	purple(Nin.)	covers No. 3
12	.50	.50	purple(Nin.)	large
13	.50	.80	purple(Nin.)	small
14	.25	.20	purple(Nin.)	long, thin
15	.25	.35	purple(Nin.)	long
16	.25	.55	purple(Nin.)	long, thin
17	.20	.75	purple(Nin.)	long, thin
18	.20	.80	yellow(U.V.)	thin
19	.15	.40	purple(Nin.)	yellow tinge
20	.10	.45	purple(Nin.)	thin

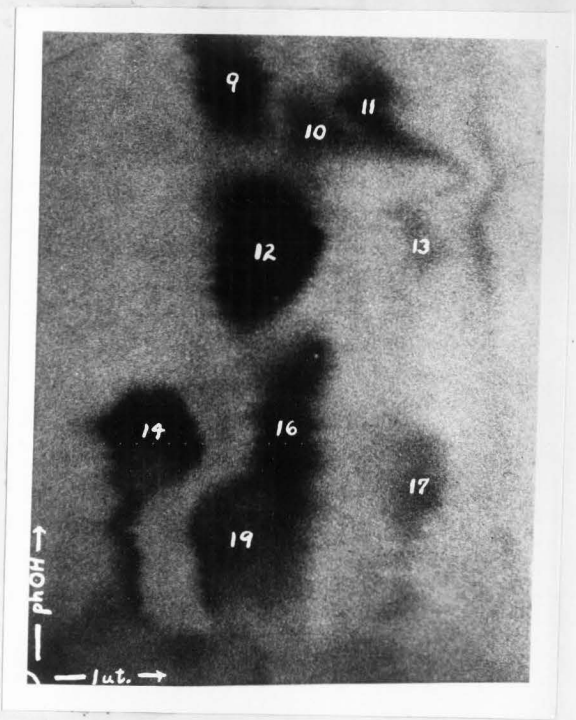

Plate 3. Example of phenol-lutidine, two-dimensional urogram with identifying numbers on visible spots

Table 17. Presence (*) or absence (-) of spots on phenol-lutidine, two-dimensional urograms from the 11th week samples from rats fed diets containing butter oil

Spot No.	Rat Number 1	2	3	4	5	6	7	8	9	10	12	13	14	15	16	17	19	20
1	-	-	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-	*
2	*	*	*	*	*	*	-	*	-	*	*	*	*	*	*	*	*	-
3	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	*	*	*	*	*	-	*	-	*	*	*	*	*	*	*	*	*	*
6	*	*	*	*	*	*	*	-	*	*	*	*	*	*	*	*	*	*
7	*	-	*	-	*	*	-	-	-	*	-	-	*	*	-	-	-	-
8	*	-	-	-	*	-	-	-	*	*	-	*	-	*	-	*	*	-
9	*	-	*	*	*	-	*	*	*	*	*	*	*	*	*	*	*	*
10	*	-	*	*	*	-	-	-	*	-	-	*	*	*	*	*	-	*
11	*	-	*	*	*	-	-	*	*	*	-	*	*	*	*	*	*	*
12	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
13	-	-	-	-	-	-	-	-	-	-	-	-	*	-	-	-	-	-
14	*	*	*	*	*	*	*	-	*	*	*	*	*	*	*	*	*	*
15	*	*	*	*	*	*	*	*	*	-	*	-	*	*	*	*	*	*
16	*	*	*	*	*	*	*	*	*	*	-	-	-	-	*	*	*	*
17	*	-	*	*	*	*	*	-	*	*	-	*	*	*	*	*	*	*
18	-	-	*	*	*	-	-	-	*	*	-	*	*	-	*	-	*	-
19	*	-	*	-	*	*	*	*	*	*	*	-	*	-	*	-	*	-
20	-	-	*	-	*	*	-	*	-	*	-	-	-	-	*	-	-	-

Table 18. Presence (*) or absence (-) of spots on phenol-lutidine, two-dimensional urograms from the 11th week samples from rats fed diets containing margarine oil

Spot No.	Rat Number 21	22	23	24	26	27	28	29	30	31	32	34	35	36	38	39
1	*	*	-	*	*	*	*	*	*	*	*	*	*	*	*	*
2	*	*	*	*	*	*	-	*	*	*	*	*	-	*	*	*
3	*	*	*	*	*	*	*	*	*	-	*	*	*	*	*	*
4	-	-	-	*	-	-	-	-	-	-	-	*	-	-	-	-
5	*	*	*	*	*	*	*	-	*	*	*	*	*	*	*	*
6	*	*	*	*	*	*	*	*	*	-	*	*	*	*	*	*
7	-	*	*	*	*	-	-	-	*	-	*	-	-	-	-	*
8	*	*	*	-	-	-	-	-	-	-	-	*	*	-	-	-
9	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
10	*	*	*	*	*	*	*	*	*	-	*	-	-	-	*	*
11	*	*	*	*	*	*	*	*	*	-	*	-	*	*	*	*
12	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
13	-	*	-	-	*	*	-	-	*	*	-	-	-	-	-	*
14	*	*	*	*	*	*	*	*	-	-	*	*	*	*	*	*
15	*	*	*	*	*	*	*	-	*	*	*	*	-	*	*	*
16	*	*	*	*	*	*	*	*	*	-	-	-	*	*	*	-
17	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
18	*	*	*	*	*	*	*	*	*	*	*	*	-	-	-	-
19	*	*	*	*	*	*	-	*	*	*	-	-	-	-	-	*
20	-	*	-	*	*	*	-	-	*	-	-	-	-	-	-	*

Table 19. Presence (*) or absence (-) of spots on phenol-lutidine, two-dimensional urograms from the 11th week samples from rats fed diets containing lard

Spot No.	Rat Number 42	44	45	46	47	49	50	51	55	56	57	58	59	60
1	*	-	*	-	*	*	-	*	*	*	*	-	*	-
2	*	*	*	*	-	*	-	*	*	*	*	*	*	*
3	*	*	*	*	*	*	*	*	*	*	*	*	*	*
4	-	-	-	-	-	-	-	-	*	-	*	-	-	-
5	-	*	*	-	*	*	*	*	*	*	*	*	*	-
6	*	*	*	*	*	*	*	*	*	*	*	*	*	*
7	-	-	-	-	-	*	-	-	*	-	-	-	-	-
8	*	-	*	*	-	-	-	-	-	-	-	-	-	-
9	*	*	*	*	*	*	*	*	*	*	*	*	*	*
10	*	*	*	*	*	*	-	-	*	-	*	-	-	*
11	*	*	*	*	*	*	*	-	*	*	-	-	-	*
12	*	*	*	*	*	*	*	*	*	*	*	*	*	*
13	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	*	*	*	*	*	*	*	-	*	*	*	*	*	*
15	*	-	*	*	*	*	-	*	*	*	*	-	-	*
16	*	-	*	*	*	*	*	-	-	*	*	*	*	*
17	*	-	-	*	-	*	-	*	*	*	*	*	*	*
18	-	-	-	-	-	-	-	-	-	-	*	-	-	-
19	-	-	-	*	-	*	-	-	*	*	*	-	-	*
20	-	-	-	-	-	*	-	-	-	-	-	-	-	-

Table 20. Presence (*) or absence (-) of spots on phenol-lutidine, two-dimensional urograms from the 11th week samples from rats fed diets containing soybean oil

Spot No.	Rat Number 61	62	63	64	65	66	67	68	69	70	71	72	73	74	76	77	78	79
1	*	*	*	-	-	-	*	*	*	*	*	*	*	*	-	-	*	-
2	*	*	-	*	*	*	-	*	*	*	*	*	*	*	*	*	*	-
3	*	*	-	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
4	*	-	-	*	-	-	*	-	*	*	*	-	*	-	-	-	*	*
5	*	*	*	*	*	-	*	-	*	*	*	*	*	*	-	-	*	-
6	*	*	*	*	*	*	*	-	*	*	*	*	*	*	*	*	*	*
7	*	*	*	-	-	-	*	*	*	*	*	*	-	*	-	-	*	-
8	*	*	*	*	-	-	-	-	-	-	-	*	-	-	-	-	-	-
9	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
10	*	*	-	-	*	*	*	*	*	*	*	*	*	*	-	-	*	*
11	*	*	*	-	*	*	*	*	*	*	*	*	*	*	-	-	*	*
12	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
13	*	-	-	*	-	*	-	*	*	*	*	*	*	*	-	-	*	*
14	*	*	*	*	*	*	*	*	*	-	*	*	*	*	*	*	*	*
15	*	*	-	*	*	*	*	*	-	*	*	*	*	*	-	-	*	*
16	*	*	*	*	*	*	*	*	*	*	-	-	-	*	*	*	*	*
17	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-	-	*	*
18	*	*	-	*	-	-	*	-	*	-	-	*	*	*	-	-	*	-
19	*	*	-	-	*	*	*	*	*	*	*	*	*	*	-	-	*	-
20	*	-	-	-	-	*	-	-	-	-	-	*	-	-	-	-	-	-

Table 21. Presence (*) or absence (-) of spots on phenol-Lutidine, two-dimensional urograms from the 11th week samples from rats fed diets containing cottonseed oil

Spot No.	Rat Number																
	81	82	84	85	86	87	88	89	90	92	93	94	95	96	98	99	100
1	-	-	-	-	*	*	-	*	*	*	*	-	*	*	-	*	-
2	-	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
3	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-	*	*
4	*	-	*	-	-	*	*	-	-	-	*	*	-	*	*	*	-
5	*	*	-	*	*	*	-	*	*	*	*	-	*	*	*	*	*
6	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-
7	-	-	*	*	-	*	-	*	*	-	*	*	*	*	-	*	-
8	*	*	-	-	-	-	*	-	-	-	-	-	-	-	*	-	-
9	*	*	*	*	*	*	*	*	*	-	*	-	*	*	*	*	*
10	-	-	*	*	-	*	*	*	*	-	*	-	*	*	*	*	-
11	*	*	*	*	-	*	*	*	*	-	*	*	*	*	*	*	*
12	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
13	*	*	*	*	-	*	*	*	*	-	*	-	*	*	*	*	-
14	*	*	*	*	*	*	*	*	*	*	*	-	*	*	*	*	-
15	-	*	*	*	-	*	*	-	-	*	*	-	*	*	*	-	*
16	-	*	*	*	*	*	*	*	*	*	*	-	-	-	-	-	*
17	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-	*	-
18	-	-	*	*	-	-	-	-	*	-	*	*	*	-	-	*	-
19	-	*	*	-	*	*	*	*	*	-	-	-	-	-	*	-	-
20	-	-	*	-	-	*	*	*	*	-	-	-	-	-	-	-	-

Although no striking differences between the five fat groups are shown by the tables, a number of interesting observations can be made. The differences which appear to be caused by dietary fat are listed below.

Differences found in the phenol, one-dimensional urograms:
1. Absence of spot No. 5 in butter samples
2. Absence of spot No. 7 in 1st week butter samples

Differences found in the propanol-ammonia, one-dimensional urograms:
1. Absence of spots 4 and 7 in animal fats

Differences found in the phenol-lutidine, two-dimensional urograms:
1. Absence of spots 4 and 13 in animal fats

Differences that were found in the two-dimensional analyses were the only ones studied further. The 9th week samples were run with phenol-lutidine and examined only for the two spots in question. For some unknown reason, a purple smear appeared in the area where spot number 13 was found. This made the examination very difficult for this spot and although the results seemed to be similar to the 11th week samples, it was felt that they could not be determined accurate enough to record. It was found that by reversing the order of the two solvents, the smear could be eliminated, although spot number 4 was lost with this method. At any rate, the 12th week samples were run by reversing the order of solvents and examined for spot number 13. A summary of the results obtained on these two spots appears in table 22.

Table 22. Percentages of 9th, 11th, and 12th week samples that contained spots No. 4 and 13 with the phenol-lutidine, two-dimensional technique

Samples	Spot Number 4	13
9th week:		
butter oil	61%	---
margarine oil	82%	---
lard	43%	---
soybean oil	89%	---
cottonseed oil	69%	---
11th week:		
butter oil	0%	5%
margarine oil	12%	31%
lard	7%	0%
soybean oil	39%	67%
cottonseed oil	53%	76%
12th week:		
butter oil	---	0%
margarine oil	---	47%
lard	---	7%
soybean oil	---	27%
cottonseed oil	---	35%

Identification of spots

Addition of Known substances. Although Rf. values obtained from urograms differed slightly from those obtained from other chromatograms, they were close enough so that by examining results of other investigations (46), some idea as to the identy of spots could be determined. These identities were checked by adding the known substances to the urine spot before chromatographing and comparing the results with the regular urogram.

Because more than one substance was thought to be present in most of the one-dimensional spots, no identification was attempted. A number of the amino acids were identified with this method in the two-dimensional series.

Table 23. Amino acids identified on the phenol-lutidine, two-dimensional urograms

Spot No.	Identity
10	valine
11	leucine
12	alanine
14	glutamic acid
15	glycine
16	serine
17	taurine

The identified spots are reported in table 23. It is quite possible that other substances are present in some of these spots, especially those covering large areas.

Selective color reagents. Twelve selective color developers, described in the appendix, were used on the phenol and propanol-ammonia, one-dimensional urograms. The spots that were found with this method are shown in tables 24 and 25. Most of these spots were not visible with either ninhydrin or ultra-violet light, so they were not examined in the first two series.

Table 24. Substances that were found with various color reagents on the phenol, one-dimensional urograms (Rf. values appear in brackets and color reagents are described in the appendix)

Color Reagent	Substances Found
AzI	none
BCG (indicator)	all acid except basic spots at Rf's (.15), (.70), and (.85)
Br_2	showed unknown white(.70), purple(.40), and yellow-blue(.10)
FCNP	gave creatinine and unknown blue-green(.20)
$FeCl_3$	showed urea and unknown gray(.40) and yellow when wet(.22) which is identical to spot No.7
HgS	none
Hyd.	tryptamine
$K_2Cr_2O_7$	urea
NHC	urea
PHC	urea
Pic.	creatinine
P-KOH	alanine and unknown light spots(.40)(.25)

Table 25. Substances that were found with various color reagents on the propanol-ammonia, one-dimensional urograms (Rf. values appear in brackets and the color reagents are described in the appendix)

Color Reagent	Substances Found
AzI	none
BCG (indicator)	all acid except basic spots at Rf's (.15), (.30), and (.60) also unknown purple spot (.80)
Br_2	histidine and unknown yellow-green-grown (.75) and white(.20)
FCNP	unknown yellow(.80) same as spot No. 2
$FeCl_3$	urea, phosphate, tartrate, and unknowns-brown(.70) and brown when wet(.75)
K_2S	none
Hyd.	tryptamine and unknown white spot(.65)
$K_2Cr_2O_7$	unknown yellow-green when wet(.80) which is the same as spot No. 2
MHC	urea and unknown rose colored spot(.50)
PHC	urea and chloride
Pic.	creatinine
P-KOH	unknown brown(.55) and black(.80) which is identical to spot No. 2

Comparison of solvents and color reagents

Many different solvents and color reagents have been reported in the literature to be useful in paper chromatography. It was thought that knowledge of their usefulness to the urographical methods herein described would be of value to further work. All of the solvents and color reagents compared are described in the appendix.

Solvents. Two dimensional urograms were used in the trials. The solvents in question were used as the second solvent to phenol and propanol-ammonia. The resulting urograms were examined under the ultra-violet light first and then treated with ninhydrin. They were judged on the number of spots separated and their distinctness. Amino acids and fluorescent compounds were judged separately because these two classes of compounds were not always separated to the same degree. The results of these trials appear in Table 26.

Color reagents. Two amino acid color reagents were used in addition to those used for identification. Isatin gives very good results with pure amino acids but only fair results on urograms. It also tends to fade quite fast. The sodium salt of 1,2-naphthoquinone-4-sulfonic acid was used in several ways. Faint blue and green colors with poor deliniation was always obtained. Neither of these color reagents is as useful as ninhydrin for urograms.

Table 26. Comparison of different solvents when used as the second solvent to phenol and propanol-ammonia in chromatographing urine (solvents are described in the appendix)

1st Solvent-	Phenol		Propanol-NH_3	
Solvent	Amino Acids	Fluor. Comp.	Amino Acids	Fluor. Comp.
α-Pic	good	good	fair	good
BuAc	fair	good	poor	fair
BuEt	poor	fair	poor	fair
BuEtAm	poor	poor	poor	poor
BuEtHCl	poor	poor	fair	good
Cre.	fair	poor	fair	poor
Et.	poor	poor	poor	poor
EtAc	fair	poor	poor	fair
EtAm	poor	poor	poor	poor
EtHCl	poor	fair	fair	fair
I-Ac	fair	fair	fair	fair
Lut.	good	good	fair	good
Me.	poor	fair	poor	poor
PhAc.	poor	poor	poor	fair
PhAm	poor	fair	poor	fair

SUMMARY

A study has been made on the effect on urinary metabolites of rats fed purified diets containing various edible fats. One hundred Sprague-Dawley, weanling rats were assigned to ten diets containing butter oil, margarine oil, lard, soybean oil, and cottonseed oil, each at 20 and 35 per cent of the diet for a 12-week period.

Growth rates showed that rats on the butter diets gained an average of 240 gms., the rats on the margarine diets gained 207 gms., lard - 201 gms., soybean oil - 215 gms., and cottonseed - 185 gms. The growth rates of rats on the 20 per cent fat diets were considerably higher than those on the corresponding 35 per cent fat diets.

At weekly intervals, the urine was collected and frozen until time of analysis. The urine was analyzed chromatographically using ascending, one- and two-dimensional chromatographical techniques. Phenol, propanol-ammonia, and lutidine were found to be the solvents which had the greatest application to this study.

A number of the spots found on the one-dimensional patterns were identified using various color reagents. Some of the two-dimensional spots were identified by adding known substances. These spots included a number of common amino acids and other common urinary constituents such as urea, creatinine, and etc.

A comparison was made of the relative usefulness of solvents and color reagents found in the literature to the urographical techniques used in this study.

CONCLUSIONS

Effects of butterfat, lard, and vegetable oils on metabolism of rats has been studied by examining metabolites excreted in the urine. Of the urinary metabolites separated, using paper chromatography, two appeared to be associated with a difference in dietary fat.

These two metabolites were not identified. Approximately 50 per cent of the urine samples from rats fed vegetable oils contained these two metabolites; whereas, they appeared in only about 5 per cent of the samples from rats fed animal fats. The importance of these unidentified compounds to the health and metabolic function of animals has not been determined. Further study is needed to characterize the metabolites and determine their role in metabolism.

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APPENDIX

APPENDIX

Chromatographing Solvents:

A number of different solvents were used in this study. They are described in approximate order of their effectiveness in separating and delineating various urinary constituents.

1. Phenol (PhOH) (48): Phenol saturated with an aqueous solution containing 6.3% sodium citrate and 3.7% potassium dihydrogen phosphate.

2. Propanol-Ammonia (P-NH_3) (9): Three parts propanol and one part 1% ammonium hydroxide.

3. Lutidine (Lut.) (48): 65 ml. 2,6-lutidine and 35 ml. water.

4. α-Picoline (α-Pic.) (12): 70% α-Picoline and 30% water.

5. Butanol-Acetate (BuAc) (36): 4 parts n-butanol, 1 part glacial acetic acid, and 5 parts water.

6. Butanol-ethanol (BuEt) (48): 80 ml. n-butanol, 20 ml. 95% ethanol, and 20 ml. water.

7. Isopropyl-acetate (I-Ac) (12): 70% isopropyl alcohol, 15% glacial acetic acid, and 15% water.

8. Cresol (Cre.) (36): 2 parts m-cresol and one part phenol solvent.

9. Butanol-ethanol-ammonia solvent (BuEtAm) (48): 80 ml. n-butanol, 10 ml. 95% ethanol, and 30 ml. concentrated ammonium hydroxide.

10. Ethanol solvent (Et) (48): 95% ethanol and 5% water.

11. Methanol solvent (Me) (48): 95 ml. absolute methanol and 5 ml. water.

12. Ethanol-acetic acid solvent (EtAc) (48): 95 ml. 95% ethanol and 5 ml. glacial acetic acid.

13. Ethanol-ammonia solvent (EtAm) (48): 95 ml. 95% ethanol and 5 ml. concentrated ammonium hydroxide.

14. Phenol-acetic acid solvent (PhAc) (48): Same as phenol solvent except that 0.5 ml. of glacial acetic acid is added to the bottom of the jar to maintain an acid atmosphere.

15. Phenol-ammonia solvent (PhAm) (48): Same as phenol solvent (No. 1) except that 0.5 ml. of concentrated ammonium hydroxide is added to the bottom of the jar to maintain an alkaline atmosphere.

16. Butanol-ethanol-hydrochloric acid solvent (BuEtHCl) (48): 80 ml. n-butanol, 20 ml. 95% ethanol, and 40 ml. 2 N hydrochloric acid.

17. Ethanol-hydrochloric acid solvent (EtHCl) (48): 80 ml. 95% ethanol and 20 ml. 0.1 N hydrochloric acid.

Color developing reagents:

The following reagents were used to develop color on the resulting chromatograms:

1. Ninhydrin reagent (Nin.) (49): Several methods were tried and the following seemed to be the most satisfactory.

A 0.2% solution of ninhydrin in acetone was poured into a shallow pan. A number of chromatograms were dipped at once then hung in a 90° to 100° oven for 5 to 7 minutes.

2. Ultra-violet light (U.V.): The chromatograms were held under the light while the spots were outlined and their colors noted.

3. Isatin reagent (Isatin) (41): Dipping into a 0.2% isatin in acetone solution containing 4% acetic acid gave different colors for amino acids after heating at 100° for 10 minutes.

4. Sodium 1,2-naphthoquinone-4-sulfonate reagent (NQS) (31): The chromatograms were dipped first into a solution containing 0.3 gm. of sodium 1,2-naphthoquinone-4-sulfonate in 10 ml. of water diluted to 300 ml. with acetone and then heated for 3-5 minutes at 80-90°. Then they were dipped into a freshly prepared solution of 2 ml. 5 N sodium hydroxide made up to 100 ml. with 95% ethanol and mixed with 30 ml. of the first reagent. They were held in the latter solution until the color intensity developed and then allowed to dry at room temperature.

5. Ferric chloride reagent ($FeCl_3$) (48): A 1% solution of ferric chloride was sprayed on lightly.

6. Alkaline ferricyanide-nitroprusside reagent (FCNP) (48): Equal volumes of 10% sodium hydroxide, 10% solutions were mixed and then diluted with three volumes of water. The mixture was allowed to stand until the dark color changed to a pale yellow before spraying.

7. Phenol-hypochlorite reagent (PHC) (48): After spraying with 5% phenol in 95% ethanol, the chromatograms were dried and sprayed with 5.25% sodium hypochlorite.

8. α-Naphthol-hypochlorite reagent (NHC) (48): A 0.1% solution of α-naphthol in 1N sodium hydroxide was sprayed on the chromatogram and allowed to dry. The second spray was composed of one volume of 5.25% sodium hypochlorite and one volume of water or ethanol.

9. Bromcresol green indicator reagent (BCG) (48): A 0.04% solution in 95% ethanol adjusted to a blue-green color with dilute sodium hydroxide was used as a spray.

10. Bromine reagent (Br_2) (48): 0.5 ml. of liquid bromine was added to 50 ml. of glacial acetic acid and 50 ml. of water. After spraying the chromatograms were heated in an oven at 90° for three to five minutes.

11. Azide-iodide reagent (AZI) (48): 50 ml. of a 0.1 N potassium iodide solution was added to 50 ml. of 95% ethanol containing 1.5 gm. of sodium azide.

12. Mercuric nitrate-ammonium sulfide reagent (HgS) (48): Chromatograms were dipped into a 0.25 M solution of mercuric nitrate in 0.5 N nitric acid and then heated for 10 minutes at 80°. They were next washed by dipping in 0.5 N nitric acid and then in water. After drying at room temperature the sheets were dipped in a solution of one part ammonium sulfide and four parts water.

13. Picric acid reagent (Pic.) (48): Chromatograms were sprayed with 0.5 N sulfuric acid and heated for one

hour at 100°. They were then sprayed with a 1.3% solution of picric acid in 95% ethanol which was combined immediately before use with one fifth its volume of 10% sodium hydroxide.

14. Hydrolytic reagent (Hyd.) (48): The same procedure was followed as with the picric acid reagent except the picric acid spray was omitted.

15. Propanol-potassium hydroxide reagent (P-KOH) (10): After dipping in propanol the chromatograms were heated at 110° for 10 minutes. Then they were dipped in 1% alcoholic potassium hydroxide and again heated at 110° for 10 minutes.

16. Potassium dichromate-formaldehyde reagent ($K_2Cr_2O_7$) (45): The chromatograms were sprayed with 9 parts 0.1% potassium dichromate and 1 part formaldehyde and then heated at 100-110° for 5 minutes.