trophoretically identical to those of 84 ml effluent could well account for the slight antibacterial activity in this fraction, in which lysozyme and ribonuclease may have exerted a synergistic effect. Nevertheless, in view of the heterogeneity of the lysosomal cationic proteins, the possibility of the existence of additional antibacterial components cannot be excluded.

The heterogeneity of ribonuclease from bovine pancreas and other sources is amply documented(12). The isoelectric points of some of these ribonucleases have been found to vary between 3 to 9.5(13,14). In our experiments the appearance of ribonuclease activity at various levels of the column indicated that the rabbit lysosomal RNAase is also heterogeneous.

The close proximity of $^{32}P$ releasing activity with antibacterial activity shows that the two activities may be identical. The concomitant disappearance of the two activities during a 19-hour electrophoretic run is another indication of their possible identity. It is significant that enzymes *per se* were incapable of invoking release of $^{32}P$ from bacterial cells, which would suggest that membrane damaging activity of cationic proteins is prerequisite for the *in vivo* degradation of phagocytized bacterial cells by lysosomal enzymes.

*Summary.* The antibacterial and enzymatic cationic proteins of acid extracts of PMN lysosomes were resolved on a preparative scale by density gradient electrophoresis. The $^{32}P$ releasing activity was closely associated with antibacterial action. Lysosomal ribonuclease was electrophoretically heterogeneous.

1. Zeya, H. I., Spitznagel, J. K., Science, 1963, v142, 1085.
2. Golub, E. S., Spitznagel, J. K., Fed. Proc., 1964, v23, 509.
3. Janoff, A., Zweifach, B. W., Science, 1964, v144, 145.
4. ———, J. Exp. Med., 1964, v120, 747.
5. Svensson, H., Analytical Methods of Protein Chemistry, Pergamon Press, 1960, vI, p225.
6. Shugar, D., Biochim. Biophys. Acta, 1952, v8, 302.
7. Schneider, W. C., Hogeboom, G. H., J. Biol. Chem., 1952, v198, 155.
8. Anderson, M. A., Szcypinski, A. J., Am. J. Clin. Pathol., 1947, v17, 571.
9. Fishman, W. H., Methods of Enzymatic Analysis, Academic Press, 1963, p869.
10. Spitznagel, J. K., Zeya, H. I., Assn. Am. Phys., 1964, v77, 126.
11. Hirsch, J. G., J. Exp. Med., 1958, v108, 925.
12. Scherga, H. A., Rupley, J. A., Adv. in Enzymology, 1962, v24, 161.
13. Sato, K., Egami, F., J. Biochem. (Tokyo) 1957, v44, 573.
14. ———, ibid., 1959, v46, 31.

Received September 9, 1965. P.S.E.B.M., 1966, v121.

## Metabolism of Isomers of 3-Hydroxykynurenine-C$^{14}$ to Quinolinic Acid, Niacin Metabolites and Carbon Dioxide.* (30750)

L. V. HANKES, R. R. BROWN† AND M. SCHMAELER
*Division of Biochemistry, Medical Department, Brookhaven National Laboratory, Upton, N. Y. and Division of Clinical Oncology, University of Wisconsin Medical School, Madison*

Hydroxykynurenine, first identified in biological sources by Butenandt *et al*(1), has since been found in a variety of sources(2,3). The enzymatic synthesis in mammals of this key intermediate in the pathway from tryptophan to niacin was first shown by De Castro *et al*(4) to occur in mitochondria of liver and kidney and to have a requirement of NADPH and $O_2$. The conversion of hydroxykynurenine to pyridine nucleotides was implied by its niacin-replacing activity in rats (5) and Neurospora(6).

The present experiments were done to study the effectiveness of keto-labeled hydroxykynurenine as a precursor of respira-

* Supported in part by U. S. Atomic Energy Commission, Am. Cancer Soc. (Grant E-202) and Nat. Cancer Inst. (Grant CA-03274).

† Supported by Research Career Development Award CA-K3-18,404, Nat. Inst. Health, U.S.P.H.S.

TABLE I. Per cent of $C^{14}$ Recovered from Rats and Mice Injected Intraperitoneally with D- or L-Hydroxykynurenine-$C^{14}$.

| | L-hydroxykynurenine-$C^{14}$* | | D-hydroxykynurenine-$C^{14}$† | |
|---|---|---|---|---|
| | Rats‡ | Mice§ | Rats‡ | Mice§ |
| $C^{14}$ injected ($\mu$c) | 1.737 | .304 | 1.811 | .453 |
| $\mu$c/g body wt | .00688 | .00585 | .00658 | .00838 |
| % in urine (0-24 hr) | 17.33 | 22.75 | 86.10 | 94.79 |
| % in $C^{14}O_2$ (0-12 hr) | 75.21 | 76.04 | 2.22 | 2.09 |
| Total % recovered | 92.54 | 98.79 | 88.32 | 96.88 |

* Specific activity 1.126 $\mu$c/mg.
† " " 1.156 $\mu$c/mg.
‡ Avg of 2 rats run individually.
§ " " 2 mice run together in the metabolism cage.

tory $CO_2$, and urinary quinolinic acid, nicotinic acid and N-methylnicotinamide in several species including the cat in which the niacin requirement is not replaced by tryptophan(7). In addition, DL-hydroxykynurenine was resolved into its D- and L-isomers and the metabolisms of these were compared in rats and mice.

*Experimental.* 3-Hydroxy-DL-kynurenine-keto-$C^{14}$ was synthesized by a method previously published(8). The D- and L-isomers were prepared by separation of the DL-mixture on a paper powder column 10 × 100 cm using as a solvent methanol, butanol, benzene, water 2:1:1:1 to which was added 2 ml of glacial acetic acid per 100 ml of solvent. This solvent mixture was previously shown to resolve DL-hydroxykynurenine into its isomers on paper chromatograms with the naturally occuring L-isomer having a greater Rf than the D-isomer(9,10). Purity of the resolved products was checked by autoradiographs of paper chromatograms and by scanning paper chromatograms with a 4 $\pi$ thin-window gas flow strip counter.

Sprague-Dawley strain rats, a common cat, a fox terrier dog and Swiss albino mice from our own colony, free of internal and external parasites, were used in these experiments. For injections into animals, saline solutions of the labeled D-, DL- and L-hydroxykynurenine-keto-$C^{14}$ were freshly prepared and administered intraperitoneally. The rats received doses of 1 to 2 ml, the dog 2.8 ml, the cat 3.8 ml, and the mice 0.35 to 0.5 ml of the saline solutions. Respiratory $CO_2$ was collected for 12 hours and urine was collected for 24 hours in a glass metabolism cage. Urinary quinolinic acid, nicotinic acid, and $N^1$-methylnicotinamide(11) were determined and isolated by carrier additions to the appropriate fractions. The isolated, purified quinolinic acid was decarboxylated stepwise in the 2 position and in the 3 position by a method previously reported(12). At the end of 24 hours the dog and cat were sacrificed and individual tissues were analyzed for carbon-14 content. The $C^{14}$ in all samples was determined by the method of Van Slyke, Steel and Plazin(13).

*Results.* As shown in Table I, the total isotope recovered in the urine and breath ranged from 88 to 99%. The rats and mice receiving the L-isomer respired about 75% of the dose as $C^{14}O_2$ compared to only about 2% of the dose of the D-isomer. In contrast only 17 to 23% of the dose of L-hydroxykynurenine appeared in the 24-hour urine while 86 to 95% of the activity in the D-isomer appeared in the urine in the same time interval. The time course of respiratory excretion for individual rats and for pooled groups of mice is shown graphically in Fig. 1 and 2. With both isomers the majority of isotope in the breath was excreted within the first 2 hours after administration, with both species excreting very similar percentages of the dose.

Data on urinary excretion of quinolinic acid, $N^1$-methylnicotinamide, and nicotinic acid by mice and rats after injection of L- or D-hydroxykynurenine are presented in Table II. L-hydroxykynurenine was a much more efficient precursor of urinary quinolinic acid than the D-isomer in both rats and mice as shown in per cent of the dose excreted as quinolinic acid and in the specific activity of this metabolite. Similarly, L-hydroxykynure-

TABLE II. 24-Hour Excretion of Quinolinic Acid (QA), $N^1$-Methylnicotinamide ($N^1$-Me) and Nicotinic Acid (NA) After Intraperitoneal Injection of D- or L-Hydroxykynurenine-$C^{14}$ (HK).

| | Hydroxykynurenine isomer injected | | | |
|---|---|---|---|---|
| | L | | D | |
| Animal species | Rats* | Mice† | Rats* | Mice† |
| Quinolinic acid: | | | | |
| $C^{14}$ in QA, $\mu$c | .0382 | .0140 | .0037 | .0014 |
| % of dose | 2.198 | 4.620 | .202 | .327 |
| Sp act, $\mu$c/mmole | 50.29 | 41.42 | 2.868 | 8.75 |
| Sp act QA/sp act HK injected | .199 | .164 | .0111 | .0338 |
| $N^1$-methylnicotinamide: | | | | |
| $C^{14}$ in $N^1$-Me, $\mu$c | .0051 | .00057 | .00064 | .00053 |
| % of dose | .293 | .188 | .0349 | .118 |
| Sp act, $\mu$c/mmole | .763 | .701 | .0782 | .905 |
| Sp act $N^1$-Me/sp act HK injected | .00302 | .00277 | .00030 | .00349 |
| Nicotinic acid: | | | | |
| $C^{14}$ in NA, $\mu$c | .00170 | .00076 | .00112 | .00026 |
| % of dose | .098 | .250 | .062 | .057 |
| Sp act, $\mu$c/mmole | .690 | .660 | .854 | .670 |
| Sp act NA/sp act HK injected | .00273 | .00261 | .00329 | .00258 |

* Avg for 2 rats.

† Pooled value for two mice.

nine was a better precursor of $N^1$-methylnicotinamide in rats than was the D-isomer. However, both isomers in both species gave about the same amount of labelling in the nicotinic acid fraction. It is doubtful if the amount of label in nicotinic acid from animals given D-hydroxykynurenine could have arisen from traces of L-isomer which might have been present in the administered preparation since the respiratory $CO_2$ data would have been more like that from L-hydroxykynurenine had there been appreciable contamination.

A comparison of the metabolism of DL-hydroxykynurenine in 4 species is shown in Table III and in Fig. 3. Data on the rat and cat were previously reported(14) but are included here for comparison and discussion. Total recovery of administered isotope in breath and urine was 83.85 to 92.02% with more than half of the activity appearing in the urine in the 24-hour period.

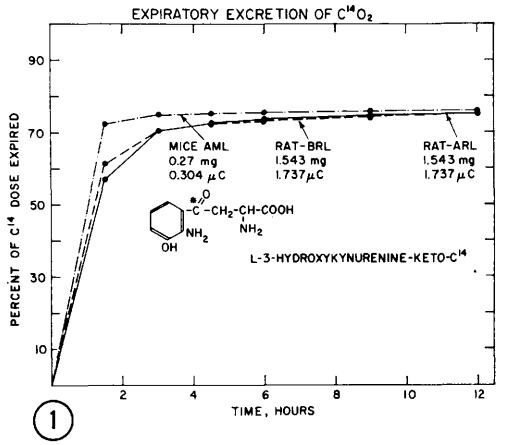
In this case, the percent of the isotope expired by the rats and mice is approximately half that found when the L-isomer was given (Fig. 1), suggesting that the D-isomer is essentially inactive as a precursor of $C^{14}O_2$ in

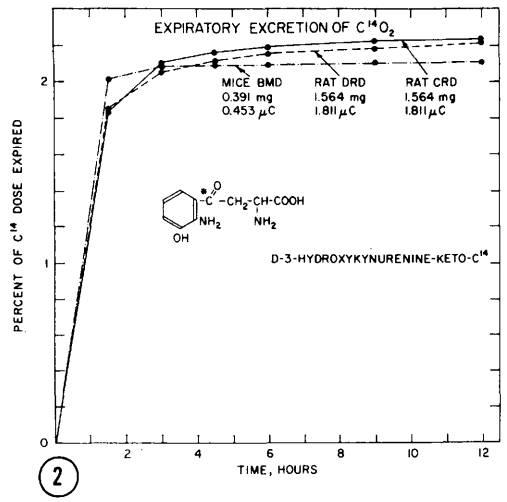
TABLE III. Excretion of Labelled Quinolinic Acid (QA) by the Rat, Mouse, Cat and Dog After Intraperitoneal Injection of DL-Hydroxykynurenine-$C^{14}$ (HK).

| Animal species | Rat | Mice* | Cat | Dog |
|---|---|---|---|---|
| Wt of animal (g) | 370 | 151 | 1413 | 1040 |
| DL-HK injected, mg | 1.01 | 2.47 | 3.952 | 2.8 |
| $C^{14}$ injected $\mu$c | 1.064 | 2.962 | 4.173 | 2.961 |
| % of $C^{14}$ in urine (0-24 hr) | 50.62 | 50.90 | 56.97 | 58.24 |
| % of $C^{14}$ in breath (0-12 hr) | 34.64 | 41.12 | 30.31 | 25.61 |
| Total recovered | 85.26 | 92.02 | 87.28 | 83.85 |
| Quinolinic acid: | | | | |
| $C^{14}$ in QA, $\mu$c | .0097 | .07 | .0984 | .180 |
| % of dose | .912 | 2.36 | 2.357 | 6.07 |
| Sp act, $\mu$c/mmole | 16.11 | 215.83 | 89.37 | 84.46 |
| Sp act QA/sp act HK injected | .0682 | .80 | .3785 | .3577 |
| % of $C^{14}$ in 2-COOH of QA | 1.32 | —† | 2.28 | 1.54 |
| % of $C^{14}$ in 3-COOH of QA | 98.68 | —† | 97.72 | 98.46 |

* Avg of 2 pooled groups of 5 mice.

† Decarboxylations were not done on quinolinic acid from mice in these experiments. However, in other experiments, quinolinic acid isolated from the urine of mice injected with DL-hydroxykynurenine-keto-$C^{14}$ contained 99.26% of the label in the 3-COOH group.



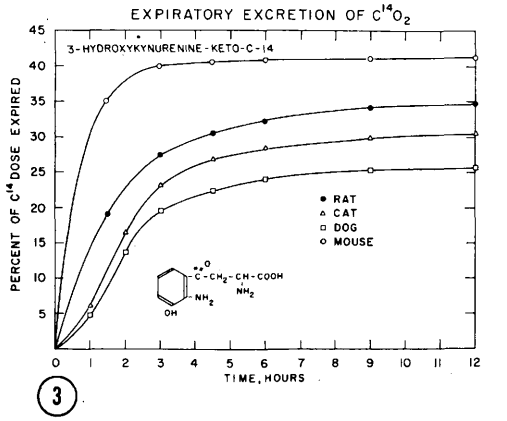




FIG. 1. Expiration of $C^{14}O_2$ by rats and mice injected intraperitoneally with L-hydroxykynurenine-keto-$C^{14}$. The curves for rats represent values from individual animals. The curve for mice is for pooled results from 2 mice.

FIG. 2. Expiration of $C^{14}O_2$ by rats and mice injected intraperitoneally with D-hydroxykynurenine-keto-$C^{14}$. The curves for rats represent values from individual animals. The curve for mice is for pooled results from 2 mice.

FIG. 3. Expiration of $C^{14}O_2$ by mice, rats and cat and dog injected intraperitoneally with DL-hydroxykynurenine-keto-$C^{14}$. The mouse and rat curves are average results from 5 and 2 animals respectively. The cat and dog values are from single animals.

these species. Further, autoradiographs of paper chromatograms of urine from mice injected with the DL-hydroxykynurenine showed most of the activity in the area of D-hydroxykynurenine (Fig. 4). Similar chromatograms from the other species also showed most of the activity in the area of D-hydroxykynurenine. However, the patterns of distribution of fluorescence and radioactivity showed differences between the species. On the basis of the experiments with D- and L-hydroxykynurenine, it is assumed that most of the urinary activity represents unmetabolized D-hydroxykynurenine.

In these experiments using DL-hydroxykynurenine-$C^{14}$ the urinary quinolinic acid was isolated by carrier procedures and from 0.9 to 6.07% of the dose administered appeared in this compound. If it is considered that the D-isomer is relatively inactive as a precursor of quinolinic acid, as is shown in Table II, then this represents a conversion of from 1.8 to 12.1% of the L-hydroxykynurenine to urinary quinolinic acid. The estimated values of 1.8 and 4.7% for the rats and mice in this experiment agree well with the values of 2.2 and 4.6 for the per cent conversion of L-hydroxykynurenine to quinolinic acid actually found in Table II.

As a check on the location of the label in the isolated quinolinic acid, the compound was decarboxylated stepwise in first the 2-COOH and then the 3-COOH. As shown in Table III, about 98% of the label was in the 3-COOH group as predicted from the position of the label in the administered hydroxykynurenine.

Comparison of the ratio of the specific activity of the urinary quinolinic acid to the specific activity of the injected hydroxykynurenine for these species shows that 6.8, 80, 38, and 36% of the urinary quinolinic acid from rat, mice, cat and dog respectively arose

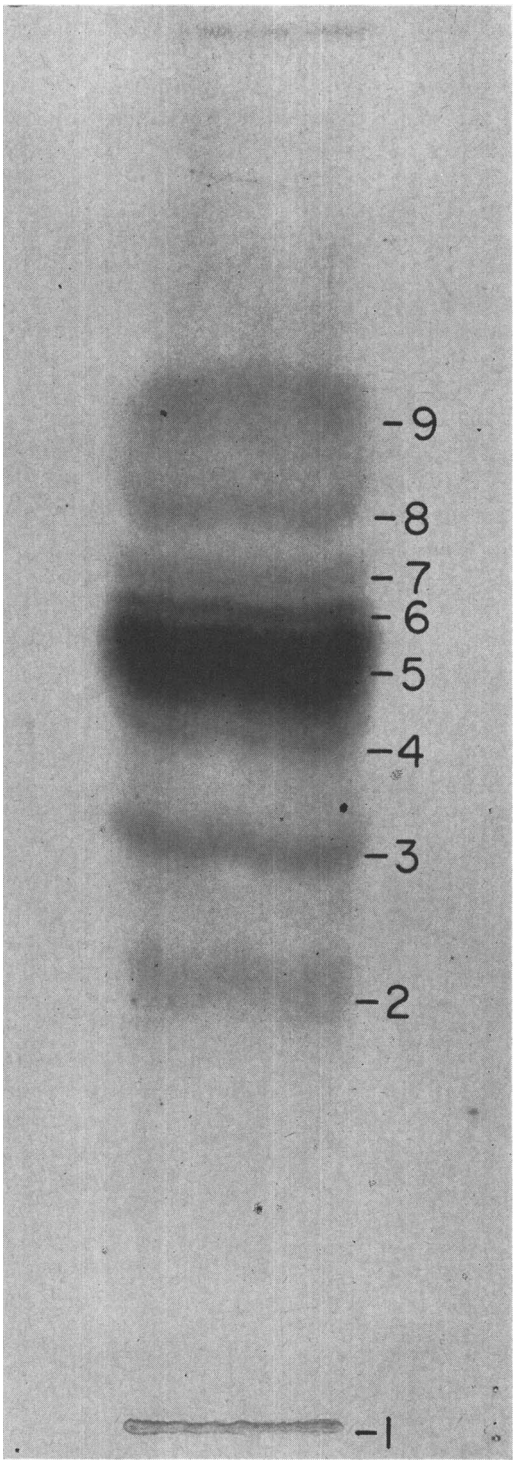



FIG. 4. Autoradiograph of paper chromatogram of pooled urine from mice injected with DL-hydroxykynurenine-keto-$C^{14}$. Spot 5 corresponds in Rf to D-hydroxykynurenine in the solvent system used which was methanol:butanol:benzene:acetic acid:water, 1:1:1:1:1 run in an ascending manner through Whatman No. 4 filter paper.

TABLE IV. Per cent of Activity Found in Tissues 24 Hours After Intraperitoneal Administration of DL-Hydroxykyurenine-keto-$C^{14}$.

| Organ or tissue | Cat | Dog |
|---|---|---|
| Liver | .665 | .382 |
| Kidney | .237 | .184 |
| Spleen | .071 | .053 |
| Heart | .024 | .000 |
| Brain | .000 | .000 |

from the injected compound. The high figure of 80% found in the mouse is in part a result of the larger dose per gram of body weight which was administered to this species.

The time course of $C^{14}O_2$ excretion following injection of DL-hydroxykynurenine-keto-$C^{14}$ is shown in Fig. 3. All 4 species metabolized the compound to $CO_2$, with most of the activity being excreted within the first 2 to 3 hours. The rat, dog and cat received the same dosage on a mg/g body weight basis and all 3 had similar excretion curves, with the dog somewhat lower and the rat somewhat higher, than the cat. The apparent high rate of excretion of $C^{14}O_2$ by the mice may be explained on the basis that the dose per gram of body weight was about 6 times that given to the other species. Previous studies from this laboratory had shown that when the dose of tryptophan per unit of body weight was increased the per cent $C^{14}$ found in the breath increased markedly(15). In the studies above (Fig. 1 and 2) with D- or L-hydroxykynurenine in rats and mice, in which the dosage rates were approximately the same, both species had very similar rates of conversion of label to $CO_2$.

Distribution of radioactivity was determined in the tissues of the cat and dog at the end of the 24-hour study (Table IV). In general, liver, kidney, spleen and heart were found to contain traces of activity amounting to less than 1% of the total dose. Brain was uniformly inactive. Skeletal muscle, serum, and blood clots from the cat contained 258, 323 and 1950 disintegrations per minute per gram wet weight of tissue in comparison with 345, 97 and 0 respectively found in the dog

samples. Total activity in these tissues could not be readily calculated due to sampling problems; however, from the above figures it seems that the blood of the cat contained considerably more activity than that from the dog.

*Discussion.* The results presented show that the naturally occurring L-isomer of hydroxykynurenine is very efficiently metabolized to $CO_2$ in contrast to the D-isomer. The small amount of $C^{14}O_2$ appearing in the breath of animals given D-hydroxykynurenine might have arisen from traces of L-isomer in the preparation. However, it is more likely that this represents non-specific breakdown of the D-hydroxykynurenine of the same order of magnitude as observed in the studies of Hankes(16) in which traces of $C^{14}O_2$ arose from administration of anthranilic acid-carboxyl-$C^{14}$ and o-aminoacetophenone-keto-$C^{14}$. The large amount of radioactivity appearing in the urine of rats and mice given the D-isomer is most probably unmetabolized D-hydroxykynurenine, since paper chromatograms and radioautographs of urine from animals given DL-hydroxykynurenine-$C^{14}$ showed that more than half of the activity was in the area of the D-isomer.

In both rats and mice, the L-isomer was much more efficiently converted to quinolinic acid than the D-isomer. However, both isomers gave about the same amount of labeling in the nicotinic acid fraction. This observation is difficult to rationalize in view of the much different labeling found in quinolinic acid. If quinolinic acid serves even indirectly as a precursor of nicotinic acid or the pyridine nucleotides(17) then labeling in nicotinic acid should have more closely followed the labeling in quinolinic acid.

In comparing the 4 species studied, the mouse, rat, cat and dog all metabolized DL-hydroxykynurenine in grossly the same manner. The differences found in the per cent of the dose converted to urinary quinolinic acid are not of sufficient magnitude to offer an explanation of why cats are not able to convert tryptophan to niacin in amounts large enough to meet their dietary needs for niacin(7). Previous work by Suhadolnik *et al*(18) indicated that metabolism of hydroxyanthranilic acid was similar in cats and rats even though *in vitro* studies showed marked differences in the conversion of hydroxyanthranilic acid to quinolinic acid and picolinic acid. They concluded that the defect in cats in the conversion of tryptophan to niacin must be before hydroxyanthranilic acid, in agreement with De Castro *et al*(19) who observed lower levels of tryptophan pyrrolase in cat liver in comparison to that found in rats. The present data in which the metabolism of hydroxykynurenine was found to be similar *in vivo* in both species suggests that the primary difference in cats probably lies prior to hydroxykynurenine in the pathway from tryptophan to niacin.

*Summary.* DL-hydroxykynurenine-keto-$C^{14}$ was synthesized and resolved into the D- and L-isomers. These were administered to rats and mice by intraperitoneal injection and urinary and respiratory radioactivity were measured. L-hydroxykynurenine was rapidly converted to $CO_2$. Urinary quinolinic acid, nicotinic acid and N-methylnicotinamide were isolated by carrier procedures and the radioactivity measured. The L-hydroxykynurenine was a more effective precursor of quinolinic acid than was the D-isomer; however, label in nicotinic acid was about the same from both isomers. D-hydroxykynurenine was not converted to $CO_2$ appreciably but was excreted probably unchanged in the urine. The metabolism of DL-hydroxykynurenine was compared in the rat, mouse, dog and cat and found to be generally the same with regard to $CO_2$ production, and urinary quinolinic acid production. No differences were observed between the cat and rats or dogs which would explain the inability of cats to utilize tryptophan as a significant source of niacin.

1. Butenandt, A., Weidel, W., Schlossberger, H., Z. Naturforsch., 1949, v4b, 242.

2. Henderson, L. M., Gholson, R. K., Dalgleish,, C. E., in Comparative Biochemistry IV, Florkin, M., Mason, H. S., Eds., Academic Press, N. Y., 1962, 288.

3. Dalgleish, C. E., Adv. Prot. Chem., 1955, v10, 81.

4. De Castro, F. T., Price, J. M., Brown, R. R., J. Am. Chem. Soc., 1956, v78, 2904.

5. Henderson, L. M,. Koski, R. E., D'Angeli, F.,

J. Biol. Chem., 1956, v223, 479.

6. Haskins, F. A., Mitchell, H. K., Proc. Nat. Acad. Sci. U. S., 1949, v35, 500.

7. Carvalho DaSilva, A., Fried, R., DeAngelis, R. C., J. Nutr., 1952, v46, 399.

8. Brown, R. R., Hankes, L. V., Kawashima, R., Biochem. Prep., 1962, v9, 79.

9. Brown, R. R., J. Biol. Chem., 1957, v227, 649.

10. Henderson, L. M., Koski, R. E., D'Angeli, F., ibid., 1955, v215, 369.

11. Hankes, L. V., Brown, R. R., Schmaeler, M., Lippincott, S., Proc. Soc. Exp. Biol. and Med., 1964, v115, 1083.

12. Hankes, L. V., Henderson, L. M., J. Biol. Chem., 1957, v225, 349.

13. Van Slyke, D. D., Steele, R., Plazin, J., ibid., 1951, v192, 769.

14. Hankes, L. V., Brown, R. R., Biochem. Biophys. Res. Commun., 1961, v4, 19.

15. Hankes, L. V., Brown, R. R., Rai, K., Fed. Proc., 1962, v21, 2.

16. Hankes, L. V., 134th Mtg., Am. Chem. Soc., Chicago, Sept. 1958, Abst., p45C.

17. Nishizuka, Y., Hayaishi, D., J. Biol. Chem., 1963, v238, 3369.

18. Suhadolnik, R. J., Stevens, C. O., Decker, R. H., Henderson, L. M., Hankes, L. V., ibid., 1957, v228, 973.

19. De Castro, F. T., Brown, R. R., Price, J. M., ibid., 1957, v228, 777.

Received September 9, 1965. P.S.E.B.M., 1966, v121.

## Bacteriophage Typing of Bacteriuric *Escherichia coli.** (30751)

WILLIAM J. BROWN† AND JOSEPH T. PARISI‡ (Introduced by C. D. Turner)
*Department of Biological Sciences, Duquesne University, Pittsburgh, Pa.*

Investigations of *Escherichia coli* bacteriuria in the last few years have shown that several serological strains are responsible for the majority of urinary tract infections(1,2,3, 4). However, these studies have been based mainly on the O group antigens, and serological identification is difficult to perform and time consuming. The purpose of this study was to determine the feasibility of identifying bacteriuric *E. coli* with bacteriophages.

*Materials and methods. Cultures.* Urine specimens were collected by the clean-catch method at Mercy Hospital, Pittsburgh, Pa., and streaked on blood agar base (Difco) containing 5% human erythrocytes. Standard dilution loops were used to determine the quantitative bacterial count on the freshly obtained specimens. Representative colonies were picked from those plates containing 100,000 or more colonies per ml of urine. Any plate showing mixed infection was discarded. Biochemical tests were performed on the isolated cultures to ascertain that the organisms were *E. coli*(5). Stock cultures were maintained by streaking the surface and stabbing the butt of tryptose blood agar base (Difco) slants.

*Isolation of phages.* Attempts to isolate phages from *E. coli* by Fisk's method(6) were unsuccessful. Therefore, 10 ml quantities of raw sewage from the Allegheny County Sanitary Authority, Pittsburgh, Pa., were inoculated into 90 ml of brain heart infusion (BHI) broth (Baltimore Biological Laboratory) and incubated at 37°C for 26 hours. Cultures were then centrifuged at 3,000 r.p.m. for 30 minutes and the supernatant fluids filtered through S1 Seitz filter sheets #6. To determine phage activity, various concentrations of filtrate and cultures of *E. coli* obtained from various sources were plated. Phage action was revealed by zones of complete or semi-complete lysis, or by discrete plaques. To distinguish between phage activity and colicin activity, the method of Bailey and Glynn(7) was used and filtrates showing colicin activity were discarded.

*Purification and propagation of phages.* Areas with discrete plaques were picked and

* Submitted by W. J. B. in partial fulfillment of the requirements for the M.S. degree.

† Present address: Dept. Microbiology, Medical Center, West Virginia Univ., Morgantown.

‡ Present address: Dept. Microbiology, School of Medicine, Univ. Missouri, Columbia.