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CATABOLISM OF DERIVATIVES OF URACIL AND CYTOSINE BY RAT TISSUES

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THE PYRIMIDINE ribosides, cytidine and uridine, have been found to be necessary for the maintenance of perfused brain (GEIGER and YAMASAKI, 1956). While surveying various rat tissues for their catabolic activities towards selected pyrimidine derivatives, we found a pattern of activities which could well contribute to the maintenance of an adequate concentration of cytidine and uridine for the brain. The catabolic responses of liver, spleen, and pancreas toward the several pyrimidine substrates studied are presented and compared with those of the brain.

METHODS

Fischer–Agouti rats (150–200 g) were fasted for 24 hr and killed by decapitation. Brain, spleen, and pancreas were homogenized in cold distilled water at a dilution of 1:10 (liver at 1:5), and the homogenates were strained through nylon tricot cloth. Substrates were obtained from Schwarz Laboratories, Niles, and prepared at a concentration of 0·007 M in 0·02 M-sodium phosphate buffer (pH 7·4) which contained 150 units of penicillin G and 300 μg of streptomycin sulphate per ml. The paper electrophoresis apparatus was essentially that described by CRESTFIELD and ALLEN (1955). All resolutions were conducted on Whatman No. 1 paper in 0·1 M-sodium tetraborate at pH 9·2 at a field strength of approximately 30 v per cm, for 45–120 min depending upon the resolution desired. With caffeine as a marker with zero charge and zero mobility, the following mobilities (cm^2 per volt per sec $\times 10^5$) relative to that for picric acid (CRESTFIELD and ALLEN, 1955) were found: caffeine (true origin), 0·0; cytosine, 0·0; applied origin (where sample was applied), 4·7; uracil, 4·9; cytidine, 6·7; uridine, 9·3; picric acid, 11·6; cytidylic acid, 12·2; and uridylic acid, 14·6. The route by which these compounds may be metabolized when used as enzyme substrates can, therefore, be easily determined, since the electrophoretic resolution is sufficient so that all probable ultraviolet absorbing catabolic products from any of the six substrates can be separately identified from their electrophoretic mobilities and confirmed by their ultraviolet absorbancy characteristics.

Technique. Buffered substrate solution (100 μl) was mixed with 50 μl of tissue homogenate (or water for controls) and incubated at 37° for 18 hr. Two volumes of 95% ethanol were added to precipitate protein and nucleic acid. After centrifugation, 100 μl of the clear supernatant fluid were applied to a tapered, pre-washed filter paper wick which stood upright in a 5 ml beaker. The dried sample was quantitatively chromatographed to the tip of the wick with the aid of 2 ml of water, and after drying, the tip was cut off and laid on the wet filter paper sheet for electrophoresis (CRESTFIELD and ALLEN, 1956); the spots were located by ultraviolet photography (SMITH and ALLEN, 1953). The spots were cut out (DAVIS and ALLEN, 1955) and treated with 4·0 ml of 0·1 M-sodium tetraborate solution (pH 9·2 with shaking for 1 hr. The optical density was read in a Beckman Model DU spectrophotometer. The recovery of the substrates after they were incubated as controls ranged from 98·2 to 101·0%, expressed as the percentage of that introduced into the reaction vessel. Thus, not only did the analytical technique appear to be satisfactory but also none of the substrates was adversely affected by the long incubation period employed in these experiments. The assays were always performed in duplicate and usually agreed within 2%. The variation from animal to animal was of course much greater. The largest individual range found was 68·2–128·9%, expressed as percentage of the average value. The average range for all of the enzyme determinations was 84·5–117·4%.

RESULTS

With uridylic acid as substrate, dephosphorylation was the first reaction and uridine was the first product (Table 1). The uridine was further metabolized to form uracil. Spleen was about nine times as active as liver and about four times as active as pancreas and brain in the dephosphorylation of uridylic acid.

All the tissues were able to form cytidine by the dephosphorylation of cytidylic acid. Pancreas, spleen, and liver were about twice as active as brain in this regard.

There appear to be two distinct enzymes for the dephosphorylation of uridylic and cytidylic acids, since the ratios of the two activitites are not constant from tissue to

TABLE 1.—THE CATABOLIC ACTIVITY OF RAT TISSUES TOWARD PYRIMIDINES

Tissue*	Substrate	First product formed†	Moles of substrate utilized per mg of dry tissue ($\times 10^9$)‡
Brain	Uracil	—	0·3§
Liver		—	2·0§
Spleen		—	0·5§
Pancreas		—	4·8
Brain	Uridine	Uracil	45·7
Liver		Uracil	94·8
Spleen		Uracil	102·2
Pancreas		Uracil	52·6
Brain	Uridylic acid	Uridine	23·4
Liver		Uridine	11·6
Spleen		Uridine	94·6
Pancreas		Uridine	23·1
Brain	Cytosine	—	0·8§
Liver		—	1·4§
Spleen		—	2·3§
Pancreas		—	1·2§
Brain	Cytidine	Uridine	7·6
Liver		—	2·7
Spleen		—	3·1§
Pancreas		—	1·0§
Brain	Cytidylic acid	Cytidine	24·8
Liver		Cytidine	64·7
Spleen		Cytidine	55·3
Pancreas		Cytidine	46·5

* Used at a dilution of 1:40 for uridine, uridylic acid, and cytidylic acid. For other substrates a dilution of 1:10 was used. Conditions of assay are further described in the text.

† (—) indicates absent or undetermined.

‡ Values are the averages of duplicate determinations on 3–5 rats, except cytosine, where only 2 rats were used. See Methods for discussion of reproducibility of replicate determinations and of the variation from animal to animal.

Because of the long incubation time used in these determinations the results are probably more representative of yield than of rate. To convert the data to moles of substrate utilized per total organ multiply by the following factors: brain, 340; liver, 1352; spleen, 73; pancreas, 88.

§ Calculated from data indicating less than 2% utilization of substrate. The activity is therefore considered to be within experimental error.

tissue. The two activities also show a different response to dilution and a difference in response to the effects of whole-body X-irradiation (SMITH and LOW-BEER, 1957).

Uridine was metabolized to form uracil at a rapid rate in all of the tissues tested. The activity of brain and pancreas was about half that found for spleen and liver.

The metabolism of [^{14}C]uracil in liver homogenates has been described (CANELLAKIS, 1956, 1957). Our data indicate that in this respect pancreas has a somewhat greater activity than liver; however, the metabolic products were not studied.

Activity of the several tissues towards cytosine appears to be absent or negligible.

A new enzymic activity has been found in brain. At variance with previous results (GREENSTEIN *et al.*, 1946), the presence of an enzyme that forms uridine by the deamination of cytidine (cytidine deaminase) has been definitely established in rat brain tissue. Uridine was identified both by its electrophoretic mobility and spectral properties. The activity was in the 105,000 **g** supernatant fluid of water homogenates and was not decreased by overnight dialysis nor increased by the addition of boiled juice. It thus appears to be a hydrolytic deaminase. The enzyme is not inactivated by freezing or lyophilization, and in this regard is similar to the enzyme found in *Esch. coli* (WANG, SABLE and LAMPEN, 1950). It is stable to heating for 1 min at 100° in the presence of cytidine, but is quickly inactivated by heating in the absence of substrate. Cytidine deaminase was also found in the brain tissue of Slonaker and Sprague–Dawley rats.

DISCUSSION

GEIGER and YAMASAKI (1956) found that perfused brain develops an abnormal carbohydrate metabolism and the period of brain survival is only about 1 hr. Inclusion of the liver in the circulation or addition of fresh liver extracts corrected this faulty carbohydrate metabolism and prolonged the survival period by several hours. When small amounts of cytidine and uridine were added to the perfusion fluid the brain was maintained in good functional condition with a normal carbohydrate metabolism for at least 4 hr without the liver (GEIGER and YAMASAKI, 1956). Uridine could not replace cytidine. One can now explain this on the basis of the present results which indicate that brain is apparently unable to convert uridine to cytidine. The reverse experiment was not performed by GEIGER to see if cytidine alone could satisfy the requirement of the brain for both cytidine and uridine. The present data suggest that this may well be possible. The relatively large capacity of liver to form cytidine from cytidylic acid and of brain to form uridine from cytidine and uridylic acid implicate these pathways as being of importance in the maintenance of an adequate concentration of cytidine and uridine in the brain.

SUMMARY

The capacities of rat brain, liver, pancreas, and spleen to catabolize the ribonucleosides and ribonucleotides of uracil and cytosine were determined by an electrophoretic method which allows the analysis of activity and identification of the products of the reaction under study to be made in one step.

Uridylic acid and cytidylic acid were dephosphorylated, by what appear to be separate enzymes, by all the tissues studied. Uridine was rapidly split to form uracil by all the tissues tested. The activities towards uracil (except pancreas), cytosine, and cytidine (except brain) under the present assay conditions were either very low or absent. Cytidine was deaminated by brain tissue.

The significance of the relatively high catabolic activities found for liver toward cytidylic acid (to form cytidine) and for brain toward cytidine and uridylic acid (to form uridine) is discussed as a possible mechanism for the maintenance of an adequate concentration of cytidine and uridine in the brain, the requirement for which has been determined in brain perfusion studies (GEIGER and YAMASAKI, 1956).

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