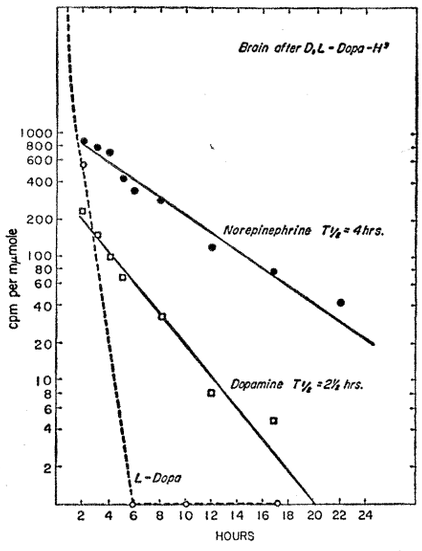

Fig. 2. Decrease in specific activities of norepinephrine, dopamine, and L-dopa in the brain following administration of tritiated DL-dopa to guinea pigs. Each point represents an individual animal.

Tritiated dopa was also found to penetrate into the brain and label the two catecholamines (Table 1 and Fig. 2). However, unlike tyrosine, dopa disappeared from the brain (and blood) extremely rapidly so that decay values for the amines could be obtained without correction for the precursor. It is of interest that approximately the same $T_{\frac{1}{2}}$ values were obtained with dopa as with tyrosine in spite of the relabeling problem associated with the latter precursor. The $T_{\frac{1}{2}}$ values obtained with H^3-labeled dopa are undoubtedly more meaningful.

From the observed $T_{\frac{1}{2}}$ values it is possible to calculate rates of synthesis in vivo. These are shown in Table 2. From the more rapid rate of turnover of dopamine and its higher concentration in the brain, one could conclude that more dopamine is synthesized and metabolized than is utilized for norepinephrine synthesis or that the dopamine pool in the brain is there not merely as a reservoir of norepinephrine precursor, but has a purpose of its own. These findings are not inconsistent with the suggestions by Carlsson *et al.* (*14*) and Sourkes (*15*) concerning a distinct role for dopamine in the central nervous system.

The calculated rates of norepinephrine synthesis in the brain of the normal guinea pig (about 0.03 to 0.04 μg/g per hr) (Table 2) compare to values of 0.05 to 0.10 reported for guinea pig heart when other procedures were used (*2, 3*). Values of this order of magnitude have also been obtained for guinea pig heart and spleen when the same precursor labeling procedure was used (*17*). By contrast, synthesis of epinephrine and norepinephrine in the adrenal gland is much more rapid. However, when values are expressed as micrograms of norepinephrine or epinephrine synthesized in the entire brain or adrenal gland of the guinea pig (Table 2), it is apparent that under basal conditions more of the hormone(s) is made centrally than in the specialized gland for epinephrine synthesis. Comparison with values obtained by similar calculations for other tissues indicates that the catecholamine synthesis in brain represents a major portion of the total catecholamine synthesis in the guinea pig. It should be pointed out that $T_{\frac{1}{2}}$ values are only for catecholamines in storage in the brain. It is conceivable that some norepinephrine or dopamine may be synthesized and metabolized without pooling with the free catecholamines which are present and extractable from the brain. If so, then these $T_{\frac{1}{2}}$ values may be too long; the actual rates of synthesis may be even more rapid than shown in Table 2. Such a mechanism was suggested as an alternative explanation for the extremely long $T_{\frac{1}{2}}$ values observed for adrenal epinephrine and norepinephrine (*18*).

The present findings indicate that norepinephrine synthesis is a rapid process in the central nervous system and further support presently held concepts concerning a neurohumoral function in the brain (*19*).

S. UDENFRIEND
P. ZALTZMAN-NIRENBERG
National Heart Institute,
National Institutes of Health,
Bethesda 14, Maryland

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Deoxycytidine in Urine of Humans after Whole-Body Irradiation

Abstract. *Increased levels of deoxycytidine were found in the urine of two humans during the first 24 hours after irradiation. This substance presumably was produced by the breakdown of DNA following irradiation, or resulted from interference with the synthesis of DNA.*

Although changes in composition of nucleotides, nucleosides, purine and pyrimidine substances have been reported in tissues and organs in several species (*1*) following irradiation of the whole body, similar changes in these substances have not hitherto been reported in humans. Increased excretion of certain amino acids in the urine for 1 to 6 days after whole body irradiation in nuclear accidents has been reported (*2*); however, we have been unable to confirm that there are any significant differences between the urinary amino acids excreted by cancer patients before or after therapeutic irradiation with cobalt-60 gamma rays, when given in doses of 50 to 200 rad over the whole body (*3*).

Parizek *et al.* (*4*) reported the excretion of deoxycytidine in the urine of rats after irradiation with doses ranging from 10 to 600 r, in amounts proportional to the radiation dosage. Zhulanova and Romantsev confirmed the excretion of deoxycytidine in the urine of rats exposed to high doses (390 to 900 r) (*5*). Later, Kosyakov *et al.* (*6*) reported increased excretion of deoxyribosides by irradiated rats (400 to 800 r) and by dogs (500 r) during the first 24

hours after radiation. In these studies, deoxycytidine was measured by the cysteine-sulfuric acid reaction described by Dische (*7*). The reaction is not specific for deoxycytidine, but measures all substances containing deoxyribose. We obtained poor recoveries in preliminary experiments in which small amounts of deoxycytidine were added to specimens of human urine. Interference resulted from the reaction of sulfuric acid with other urinary constituents and with pigments and medications.

Nucleic acid derivatives were isolated and concentrated by absorption on charcoal and elution with pyridine (*8*). The solvents used to separate the nucleic acid derivatives on paper chromatograms were AmSu (*8*), BuA (*8*), and INH (isopropanol, 80; concentrated ammonium hydroxide, 10; water, 10). Both deoxycytidine and thymidine can replace vitamin B_{12} in the nutrition of *Lactobacillus leichmannii* 313 (*9*). These substances were assayed on nutrient agar containing the constituents of the medium used for assay of B_{12} and seeded with the organism (*10*). Strips of chromatograms run in AmSu, BuA, or INH were placed on the seeded agar and, after incubation, growth zones corresponding to deoxycytidine and thymidine were measured.

Deoxycytidine was recovered from urine of rats after total body irradiation with 760 r. Peak excretion occurred during the first 24 hours (6 to 7 mg/24 hr) and none was detected after 72 hours. Thymidine, present in urine specimens obtained before irradiation (0.1 mg/24 hr), was increased in specimens obtained after irradiation (2.5 mg/24 hr).

These data confirm the findings of other investigators regarding the excretion of deoxycytidine in the urine of rats after irradiation. Parizek also noted increased excretion of thymidine by rats following irradiation (*4*). He reported values for deoxycytidine and thymidine in the same range as those we found.

To determine if the same effect could be noted in humans, we tested the urine of patients receiving therapeutic irradiation of the whole body for malignancies. Radiation was delivered by a cobalt-60 teletherapy unit; half the specified exposure was delivered laterally through one side, after which the patient was turned and the other half was delivered laterally through the other side. The dose rate in air, as measured with a Victoreen 25-r high-energy chamber was 5.4 r/min. Specimens of urine were collected from each of two cancer patients at three 24-hour intervals before irradiation. The patients were exposed to the appropriate midline air dose (238 r and 257 r for the two patients reported here) to result in a midline absorbed dose of 150 rad. Following irradiation, the total volume of urine was collected at 6-hour intervals for the first 48 hours. For the 3rd, 7th, 10th, and 13th days after irridation, collections were again made at 24-hour intervals. Specimens were collected in containers kept in ice, and were frozen immediately afterward until they were analyzed. Ten milliliter aliquots were used for the assays of deoxycytidine and thymidine. The minimum amount of deoxycytidine which could be detected in the urine was approximately 4 μg/ml.

Table 1. Deoxycytidine and thymidine in the urine of two humans before and after irradiation.

Collection No.	Patient No. 1 (male)		Patient No. 2 (female)	
	Deoxycytidine (mg/6 hr)*	Thymidine (mg/6 hr)*	Deoxycytidine (mg/6 hr)*	Thymidine (mg/6 hr)*
Control period (collection every 24 hours)				
1	—	—	—	—
2	—	—	—	—
3	—	—	—	—
Post irradiation period (collection every 6 hours)				
1a	35	34	20	—
1b	47	35	15	—
1c	23	14	6	—
1d	5	7	11	—
2a	31	8	—	—
2b	30	38	—	—
2c	11	20	—	—
2d	†	†	—	—
Subsequent collections (every 24 hours)				
3	—	—	—	—
7	—	—	—	—
10	—	—	—	—
13	—	—	—	—

* A dash indicates that, if present, the amount was below the minimum detectable (0.04 mg in 10 ml). The volume of specimens taken at 6-hour intervals was 150 to 500 ml. † Specimen lost.

Chromatograms were obtained which showed several conspicuous ultraviolet-absorbing spots which were clearly separated from deoxycytidine and thymidine in the solvent systems used. The patients were usually given sedating medications, many of which absorb in the same wavelength and which could then be identified by other chemical reactions. Deoxycytidine was not detected in specimens collected before irradiation. The rates of excretion of deoxycytidine and thymidine by two humans are shown in Table 1. In one patient, deoxycytidine was excreted during the first 48 hours after irradiation; maximum excretion occurred during the first 12 hours. In a second patient, deoxycytidine was excreted during the first 24 hours only. Thymidine was present in urine specimens from patient No. 1 during the first 48 hours after irradiation; it was not detected in the second patient at any time. Deoxycytidine could not be demonstrated in specimens from control patients with cancers treated surgically or from a patient with severe burns.

The nucleic acid concentrates from human urine were tested by the Dische reaction. Specimens obtained the day before irradiation, as well as 1, 2, and 3 days after irradiation, were strongly positive. The apparent deoxyriboside content of the urine extracts, measured by the Dische reaction, was approximately ten times greater than that of the combined deoxycytidine and thymidine content measured after chromatographic separation.

The use of the nonspecific cysteine-sulfuric acid reaction may account for the observation by Kosyakov that a broad range of deoxyribose-containing substances were excreted by both well and sick human subjects (*6*). The human urine which we examined contained other ultraviolet-absorbing substances, as well as other Dische-positive substances. Fractionation of the nucleic acid derivatives on paper chromatograms was necessary to demonstrate the excretion of deoxycytidine by human beings following irradiation (*11*).

HELEN K. BERRY, EUGENE L. SAENGER
HAROLD PERRY, BEN I. FRIEDMAN
JAMES G. KEREIAKES, CAROLYN SCHEEL
Departments of Radiology, Pediatrics, and *Internal Medicine* and *Children's Hospital Research Foundation, University of Cincinnati College of Medicine, Cincinnati, Ohio*

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Electrophoretic Patterns of Hemoglobin from Fetal Mice of Different Inbred Strains

Abstract. *Starch gel electrophoretic patterns of hemoglobins from fetal mice of seven inbred strains (two with single, five with diffuse adult hemoglobins), were compared with each other and with electrophoretic patterns of hemoglobins from adults of the same strains. The blood from 15-day-old fetuses of all strains contained four electrophoretically separable heme components. However, there seems to be a difference between strains with single and diffuse adult hemoglobin in the time of emergence of a clear adult pattern.*

Two electrophoretically distinct types of hemoglobin are known in adult mice of different inbred strains (*1*). Electrophoretic studies with various supporting media have served to demonstrate inherited strain specificity for either the single (discrete single band) or the diffuse (two or more bands) type of hemoglobin (*2*). The difference between these patterns is controlled by a single pair of genes (Hb^s, single; Hb^d, diffuse). Recently, two reports have been published which describe the electrophoretic nature of mouse fetal hemoglobin (*3*, *4*). Additional components that contained heme, which are presumed to be fetal hemoglobin, were noted in early fetal mice of both the C57BL/6J strain (Hb^sHb^s) and the CBA strain (Hb^dHb^d). The concentration of these additional hemoglobins decreased steadily throughout fetal life and they were entirely absent at birth. Since the studies with these two inbred strains were carried out separately, no effort was made to compare the hemoglobins from Hb^sHb^s and Hb^dHb^d fetuses. The present experiments were conducted to determine whether or not electrophoretic differences can be detected when fetal hemoglobins from strains with single and diffuse adult hemoglobins are run side by side on a starch gel.

Mice from two Hb^sHb^s strains (C57BL/6J, SEC/Re) and five Hb^dHb^d strains (FL/Re, AKR/J, 129/J, DBA/2J, and CBA/J) were used. Data were collected from a minimum of five gels per strain and, in most cases, fetal hemoglobins from two or more Hb^dHb^d strains were placed on the same gel, together with their adult controls and a C57BL/6 sample of the same age.

Fetuses aged 15 days (from the day on which vaginal plugs were observed) were selected for use in screening the different strains because, at this stage, one litter was generally sufficient to ensure the collection of enough cells for hemoglobin testing while fetal components were still in evidence. The collected cells (*4*), usually 0.05 to 0.1 ml, were washed once by suspension in 10 ml of cold saline and sedimented in a clinical centrifuge at high speed for 15 minutes. The supernatant was drawn off and the cells were lysed in three times their volume of distilled water. The precipitation of stroma was effected by the addition of a salt solution (1.0*M* NaCl, 0.07*M* $MgCl_2$) in an amount equaling 15 percent of the total volume of lysate. Precipitated stroma were sedimented in a Servall Superspeed Centrifuge for 30 minutes at 15,000 rev/min, and the clear hemoglobin solutions decanted and converted to carboxyhemoglobin. All procedures, except the addition of CO, were carried out in the cold, and solutions were placed on a gel within 24 hours of sample collection. Separations were accomplished by starch gel electrophoresis using the discontinuous buffer system of Poulik (*5*), run for 2½ to 3 hours at approximately 5 volt/cm. Following the completion of a run, the gel was sliced and stained with Amido Black 10B (see *6*) or, less frequently, with 3,3′-dimethoxybenzidine.

When prepared by these methods, solutions from adults with single hemoglobin give a single band while those from adults with diffuse hemoglobin give two separable bands of unequal concentration with the highest concentration in the more rapidly moving component. Two additional characteristics of Hb^dHb^d hemoglobins (both fetal and adult) in these preparations are a tendency to streak in the medium and the appearance of an area projecting ahead of the major component which does not resolve to give a discrete band (Fig. 1). The number and mobilities of fetal components appear to be similar in preparations of Hb^dHb^d and Hb^sHb^s fetuses (Fig. 1). Fetuses of Hb^sHb^s strains produced a pattern consisting of four bands with a very high concentration in the most rapidly moving band, giving the hemoglobin pattern of these fetuses at 15 days' gestation an appearance close to that of an adult. The concentration of fetal components varied from litter to litter in all Hb^dHb^d strains tested. However, there was not the great resemblance to the adult pattern frequently observed in 15-day Hb^sHb^s fetal preparations. Fetal preparations from Hb^dHb^d strains showed a single band in the region of the adult major component, a second band in the region of the adult minor component, and two additional bands, which resembled the third and fourth components of Hb^sHb^s fetuses in their mobilities.

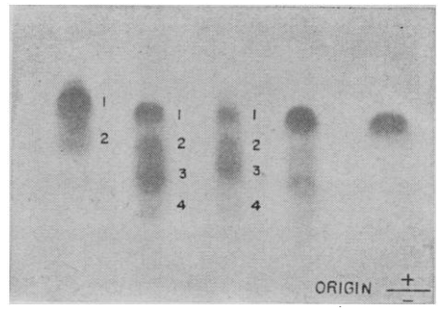

Fig. 1. Fetal and adult hemoglobin components on starch gel developed by staining with Amido Black 10B. Additional protein bands (*6*) which are also developed with this stain are not visible in this photograph. Left to right: AKR (adult), C57BL/6 (from 13-day fetus), AKR (14-day fetus), C57BL/6 (14-day fetus), C57BL/6 (adult).

To test the reality of apparent differences in maturity between the Hb^dHb^d and Hb^sHb^s fetal hemoglobin patterns, preparations from 14-day fetuses of one of the Hb^dHb^d strains (AKR) were placed on gels together with preparations from 13-day and 14-day fetuses of one of the Hb^sHb^s strains (C57BL/6). The results are shown in Fig. 1. The bands have been numbered for reference, beginning with the one nearest the anode. In AKR preparations it could be noted that band 3 had the highest concentration at 14 days. Bands 1 and 2 appeared to be in approximately equal concentration with respect to one another. In 14-day