SPECIES DIFFERENCES IN DESOXYCYTIDINE METABOLISM

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The author has previously shown that, in contrast to rats, in other laboratory animals (mice, dogs, rabbits) the nucleoside of desoxycytidine (DC) is present in the urine in very small amounts, or cannot be detected at all [3]. The action of radiation, even if massive (in absolutely lethal doses), causing a 20-30-fold increase in the level of DC excretion in rats [1], does not produce any marked increase in excretion of the nucleoside in these animals.

At the same time, there are no grounds for assuming any species differences in the DC whether at the molecular (structure and function of DNA) or at the cellular level. It was postulated, therefore, that these species differences are due to differences in the enzyme apparatus of some organs of rats by comparison with animals of other species.

To test this hypothesis, the internal organs of rats, rabbits, and mice were investigated to determine their metabolic activity in relation to DC. The other purpose of the investigation was to determine the metabolic "fate" of this nucleoside in animals not excreting it, because this could be useful in seeking a metabolite important for testing for a radiation injury in such animals.

The experimental animals were noninbred dogs, rabbits weighing 2.0-2.5 kg, noninbred rats and rats of the Wistar line weighing 200 g, and laboratory mice weighing 15-20 g. The animals were killed by decapitation (except for the dogs, which were electrocuted). The liver was washed with cold (2-4°) physiological saline, and the remaining organs (kidneys, spleen, heart) were freed from visible blood clots. From each tissue, two equal samples were taken (for the experimental and control tests): for the liver the sample weighed 2 g and for the other organs between 0.8 and 2.0 g. The small organs (spleen, kidneys of the rats and mice) from a few animals were pooled. The sample of tissue was ground in a mortar with powdered glass and suspended (1: 2) in Krebs-Ringer solution containing polymyxin (1000 units/ml) for physiological saline. For the experimental samples with the addition of DC, the added solutions also contained DC in a concentration of 70 μg/ml.

Besides the tests with the homogenates in each series control tests were carried out with physiological saline instead of homogenates; physiological saline was taken in the same volume as in the ordinary test: 2 ml to 4 ml of the corresponding solution (with and without addition of DC). The samples were incubated at 37° for 1.5 h with agitation. After incubation, 10% TCA solution was added to give a final concentration of 4%, and after standing for 10-15 min in the cold, the residue was removed by centrifugation. The DC concentration in the supernatant fluid was determined by a method proposed earlier [2], but without the stage of methanol extraction.

The decrease in the added DC during incubation of the homogenate of the organ was determined as follows: The amount of DC remaining in the sample after incubation was found from the difference between the DC concentration in the experimental sample (homogenate and solution with the addition of DC) and in the corresponding sample without the addition of DC (control). The difference between the amount of DC determined in the corresponding control sample and this last value represented the decrease in added DC as a result of incubation of the homogenate. This decrease was expressed as a percentage, taking the content of DC obtained in the control sample as 100.

EXPERIMENTAL RESULTS

The results showing the decrease in added DC during incubation of homogenates of the organs of rats and rabbits are given in the table. They show that the kidney and spleen tissue exhibited no significant species differences. The decrease in the added DC in these organs was relatively high.

At the same time, marked species differences were found in the liver tissue. The relative activity of the rats' liver was approximately one-tenth that of the rabbit's liver. In the liver of the mice, and also of the rabbits, a relatively high rate of DC metabolism was found. During incubation of the mouse liver homogenate for 90 min, the

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Decrease in Added DC during Incubation of Homogenates of Organs from Animals of Different Species in Normal Conditions (in Percent of Amount Added to Sample)

Organ	Rat	Rabbit	Mouse	Dog
Liver	80 (72—140)	17 (12—24)	0—7	0—8
Kidney	28 (0—64)	22 (7—65)	0—15	30
Spleen	30 (18—36)	16 (0—32)	—	74

Note: Five experiments each were carried out with the organs of the rats and rabbits, three experiments each with the organs of the mice and dogs in the case of the liver and one or two experiments for the other organs.

decrease in added DC was 93-87% compared with 7-21% in the parallel experiments with rats' liver homogenates. A similar picture was obtained in the experiments with dogs' liver homogenates (see table).

To confirm that the observed decrease in DC in the experiments with the liver homogenate was due to the enzyme activity of the tissue and not to incidental factors (sorption), parallel with the ordinary tests, control tests were carried out on samples of the same composition but without incubation. In these samples, the decrease was relatively small: the difference between the control samples with the tissue and the samples with physiological saline (instead of tissue) was 10-20%. Hence, it follows that the decrease in DC in the experiments described above was due mainly to the enzyme activity of the tissue.

The differences discovered between the enzyme activity of the liver of the rat and the other species of animals were evidently attributable to the high level of excretion of DC. It may be supposed that the DC entering the blood stream from the different organs and tissues (mainly the actively proliferating organs) is metabolized by the liver in animals such as the dog, rabbit, and mouse. In rats this is impossible, and this evidently is responsible for the exceptionally high level of DC in the tissues of rats by comparison with animals of other species [4] and for its increased excretion.

It can be considered that the species differences observed in the enzymic apparatus of the liver in rats are also responsible for the sharp increase in the excretion of DC produced in these animals by the action of ionizing radiation. As shown by experiments on the organs of irradiated animals, the same relationships are observed in the levels of enzyme activity in such animals as in normal conditions. For instance, the decrease in the added DC during incubation of liver homogenates of animals of different species after irradiation in a lethal dose (in percent of the amount added to the sample) was as follows: 80-58 (rat), 24 (rabbit), 0-15 (mouse), and 1-12 (dog). Two experiments were carried out with the liver of each species of animal (except the rabbit). The animals were irradiated with a minimal absolutely lethal dose: 700 R (rabbits, rats, and mice) and 400 R (dogs). After the level of the metabolic activity of the liver of the rabbits and mice in relation to DC had been shown to be high, an attempt was made to discover the pathway of its conversion in this organ. Following a recently published report of the relatively high activity of DC deaminase in mouse liver [4], it was suggested that the detachment of the amino group catalyzed by this enzyme is the first stage in the metabolism of this nucleoside in the liver of the mice, rabbits, and dogs.

To test this hypothesis, a TCA-filtrate containing DC and the products of its metabolic conversion was fractionated on KU-2 resin in the H^+-form. During fractionation of this type, as special experiments by the author showed, the pyrimidine compounds not possessing amino groups (uridine, thymidine) underwent practically no absorption on the resin and remained in solution.

The results of Dische's reaction with the TCA-filtrate, passed through the cation-exchange resin, revealed a high concentration of DC in the liver samples of the dog, rabbit, and mouse and a very low concentration of DC in the liver samples of the rats. For instance, in one typical experiment, the following amounts of Dische-positive substances were found in the TCA-filtrates (in percent of added DC): 7 (rat), 132 (rabbit), 110 (mouse); it may be assumed that the reason for the "rediscovery" of Dische-positive substances in the TCA-filtrate was the difference in the coefficients of molar extinction of DC and of the compound formed from it, and also the nonstandard conditions in which the reaction was performed (the test was carried out with 0.5 ml of TCA-filtrate instead of water).

These results are in good agreement with the hypothesis described above suggesting that deamination is an early stage in the conversion of DC in the liver of mice, rabbits, and dogs. At the same time, the results obtained are not strict proof that this is in fact the pathway of DC metabolism, because a similar picture during fractionation on an ion-exchange resin would be observed if the DC were split into pyrimidine and carbohydrate components (for example, by the action of nucleoside phosphorylases).

To exclude this possibility, metabolites of DC were fractionated by the method of chromatography on paper. For this purpose the TCA-filtrate, having passed through a column of KU-2, was repeatedly extracted with ether to remove the TCA, evaporated in vacuo to a small volume, and applied to the chromatogram. After development of the chromatogram in a system of ethyl acetate—water—formic acid (60: 35: 5), the localization of desoxyribose on the chromatogram was revealed by Dische's method. These experiments showed that the value of R_f for the compound concerned (about 0.15) and for desoxyribose (the standard 0.5) did not coincide. These results show that during incubation of DC, this substance probably is not split into a base and a carbohydrate component, and they indicate that deamination is the main pathway of conversion of DC in the liver tissue of dogs, rabbits, and mice.

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