# Relative Dehydrogenase Activity of Kidney and Liver Slices from Control and Tumor Mice.* (20096)

MAURICE M. BLACK AND FRANCIS D. SPEER.
(With the technical assistance of Phyllis Brenowitz.)
*From the Department of Pathology, New York Medical College, Flower and Fifth Avenue Hospitals, New York City.*

In a previous study we had employed the *in vitro* reduction of 2,3,5-triphenyltetrazolium chloride (TTC) to evaluate the dehydrogenase activity of liver and kidney slices in control and tumor bearing mice(1). The data obtained did not indicate any striking difference in the dehydrogenase activity of liver or kidney slices from tumor mice. In later work on this point and in a review of the aforementioned data it was noted that the kidney to liver ratio (K/L) of the endogenous dehydrogenase activity/mg tissue was lower in some strains of tumor bearing mice than in the controls of the same strain.

We therefore undertook an investigation of the K L ratio in mice of different strains with and without spontaneous mammary tumors. The present findings indicate that the relative intensity of the dehydrogenase activity of kidney and liver slices tends to be maintained within a relatively narrow range in the groups studied. This K/L ratio is shifted in the majority of tumor mice of the CFW and A strains to a lower value. Such changes may occur in animals with very small tumors and does not appear to be related to inanition or cachexia. In addition, there appears to be a relationship between the K/L ratios in the control mice and the appearance of spontaneous tumors within the strain.

*Materials and methods.* The control mice included adult virgin females of the CFW, A, dba, and C3H strains. The tumor animals were female CFW, A, dba, and C3H mice bearing spontaneous mammary carcinomas. The animals were sacrificed by crushing the cervical cord and the kidneys and livers were quickly excised. Slices of approximately 1 mm in thickness were prepared from the left lobe of the liver and the mid-portion of the kidney. The kidney sections being sagittal included both cortical and medullary tissue. The tissue sections were placed in wide mouthed test tubes containing 3 ml of 1% TTC and 1 ml of 0.9% saline, buffered to pH 7.2. The tubes were placed in an incubator maintained at 37°C and subjected to continuous gentle agitation for one hour. Under these conditions the water soluble colorless TTC is reduced enzymatically to a red water insoluble formazan. At the end of this time the solutions were decanted and the reactions stopped by fixing the tissues in 10% neutral formalin. The formazan deposited in the tissues was then extracted with repeated washings of acetone and the total volume made up to 5 ml. The color intensity was determined in a Coleman spectrophotometer set at 470 mmu. These readings were converted to $\mu$g of TTC reduced by the use of a calibration curve obtained by the reduction of known amounts of TTC with crystals of sodium sulfide. The concentrations/ml obtained, which correspond to the values previously reported(1,2) were multiplied by the volume, 5 ml to yield the total amount of TTC reduced. The acetone dried tissues were weighed on a Roller-Smith microbalance (0-50 mg) and the $\mu$g TTC reduced/mg tissue was determined. All determinations were run in duplicate. These values are taken to be indicative of the endogenous dehydrogenase activity ($R_O$).

*Results.* In Table I we have listed the pertinent findings obtained. It will be noted that there is a tendency for the $R_O$ value for the liver slices from the tumor animals to be somewhat greater than those of the control animals in the CFW, A, and dba strains. At the same time, the $R_O$ values for the kidney slices from the tumor animals tended to be somewhat lower than those of the control animals in the CFW, A, and C3H strains. However, the magnitude of these changes is

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TABLE I. Endogenous Dehydrogenase Activity of Kidney and Liver Slices from Control and Tumor Mice of Different Strains.

| Strain | No. | Body wt (g) | Liver $R_o$ | Kidney $R_o$ | $R_o$ K/L | P† |
|---|---|---|---|---|---|---|
| CFW | | | | | | |
| T* | 40 | 35.0 ± 3.81 | 21.53 ± 3.66 | 31.71 ± 3.99 | 1.51 ± .29 | <.01 |
| C* | 28 | 28.2 ± 5.21 | 18.15 ± 1.9 | 33.24 ± 2.94 | 1.84 ± .22 | |
| A | | | | | | |
| T | 13 | 28.9 ± 3.78 | 22.2 ± 3.82 | 36.6 ± 2.24 | 1.70 ± .31 | <.02 |
| C | 18 | 21.6 ± 2.86 | 19.3 ± 3.9 | 38.0 ± 4.9 | 2.01 ± .31 | |
| dba | | | | | | |
| T | 13 | 28.3 ± 3.41 | 22.8 ± 3.8 | 39.8 ± 4.7 | 1.77 ± .24 | >.05 |
| C | 23 | 22.3 ± 3.11 | 17.85 ± 2.8 | 34.85 ± 5.1 | 1.98 ± .33 | |
| C3H | | | | | | |
| T | 30 | 29.4 ± 3.82 | 20.56 ± 3.5 | 33.89 ± 4.14 | 1.69 ± .35 | >.57 |
| C | 32 | 23.9 ± 3.58 | 20.79 ± 3.39 | 35.12 ± 2.85 | 1.74 ± .34 | |

* T = Tumor. C = Control.

† P = Probability that difference in the K/L ratios between control and tumor group is due to chance variation.

far from decisive. On the other hand, more significant differences are found when a comparison is made of the K/L ratios obtained in the tumor and control mice of the CFW and A strains. The K/L values listed in Table I represent the average values and standard deviations of the ratios of the individual mice in the group. The range of these values in each group is in the form of a normal distribution curve. It will be noted that the K/L ratios of the tumor mice of the CFW and A strains are significantly lower than those of the control mice of the same strains. On the other hand, no such difference is observed between the tumor and control group of the C3H mice. In the case of the dba mice the difference in the K/L ratio between the control and tumor groups is just below the level usually considered statistically significant.

In order to determine whether the size of the tumor was a factor in the observed changes in the K/L ratio, a comparison was made of the relationship between the tumor size and the K/L ratio in the CFW tumor mice. This group was divided in two: those with tumors weighing less than 0.6 g and those with larger tumors. In the group with the smaller tumors the mean K/L ratio obtained in 22 mice was 1.49 ± 0.27. The mean value in 18 mice with larger tumors was found to be essentially the same, *viz.* 1.53 ± 0.32.

Since the tumor bearing mice are heavier than the controls it is pertinent to question whether the K/L ratios might vary with body weight rather than with the presence or absence of a tumor.

If the difference in body weight were the critical variable that accounted for differences in the K/L ratios between the tumor and control animals then one would expect to find an inverse relationship between the K/L ratio and the weight of the tumor animal. To test this possibility we have divided the CFW tumor animals into 2 groups: those weighing <35 g and those weighing >35 g. The mean weights and K/L ratios in these 2 groups were found to be: body weight 32.5 g, K/L ratio 1.42 and body weight 38.0 g. K/L ratio 1.69. It is thus evident that the K/L ratio does not vary inversely as the body weight.

*Discussion.* A variety of clinical and laboratory studies have suggested that alterations in liver function and enzymatic activity occur in tumor bearing animals and patients(3-5). In addition, changes in the enzymatic activity of the kidney in the tumor host have been reported in regard to d-amino acid oxidase and arginase(4). Further evidence of a change in kidney function in cancer patients is suggested by the report of Reifenstein *et al.*(6) on the response of cancer patients to ACTH. They found that as a group, cancer patients showed a normal response after ACTH in regard to the fall in circulating eosinophils and the rise in the urinary K/creatinine ratio. However, as a group they showed subnormal changes in regard to the expected rise in urinary P/creatinine and uric acid/creatinine ratios. It will

be noted that these findings reflect not only an adrenal defect but also an alteration in renal function. In view of the above observations on hepatic and renal function in the tumor host, it might be expected that decided changes in the dehydrogenase activity of these tissues would be found. While our data indicate a tendency for the dehydrogenase activity of the kidney slices to fall and the liver slices to rise in the tumor group as compared with the controls, these changes are of limited magnitude and the overlap of the absolute values in the 2 groups is considerable.

The present findings are of interest from several aspects: (a) In view of the importance of the homeostatic functions of the liver and kidney(7) and clinical observations linking kidney and liver function; *i.e.*, the hepatorenal syndrome, it is significant to find evidence of a relatively fixed relationship between the dehydrogenase activity of the liver and kidney. (b) The fall in the K/L ratio in the tumor mice of the CFW and A strains is indicative of a systemic alteration in the tumor host. Since this change was found in the mice with small as well as large tumors, it more probably represents an effect of the tumor rather than an expression of cachexia or inanition. (c) The findings are of interest in regard to the incidence of spontaneous tumors within the strains. The most marked differences in the K/L ratios between the control and tumor mice were found in the CFW and A strains. These strains are known to have a very low incidence of spontaneous breast tumors in the virgin state. In contrast, the K/L ratios in the control and tumor mice of the C3H strains were almost identical. This strain is characterized by a very high (90%) incidence of spontaneous tumors in the virgin mice. The dba strain has an intermediate incidence of breast tumors in the virgin females (11-50%). In this strain the difference in the K/L ratios between the control and tumor animals was found to be just below the level of statistical significance.

*Summary.* The *in vitro* dehydrogenase activity of liver and kidney slices from female control and tumor mice of the CFW, C3H, dba, and A strains was determined and the relative intensity of the kidney to liver values (K/L ratio) was calculated. The K/L ratio was found to be maintained within a relatively narrow range in the different control groups. In the CFW and A mice this value was shifted to a lower value in the presence of a tumor. Such a change occurred to a lesser degree in the dba mice and not at all in the C3H mice.

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## A Plasminogen Activator in Spontaneously Active Human Blood.*† (20097)

STEN MULLERTZ. (Introduced by T. Astrup.)

*From the Biological Institute of the Carlsberg Foundation, Copenhagen, Denmark.*

In the living organism fibrinolytic activity of blood occurs in a number of physiological and pathological conditions(1,2). The high activity found after certain modes of death (3) has recently been studied(4). The specific fibrinolytic effect observed in blood is caused by a strong adsorption of the fibrinolytic substances to fibrin(5). The experi-

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