by inoculation into mouse embryo cultures or into mice with completely negative results. When they were respectively 40, 30 and 23 days old, passage 2, 3 and 4 subcultures of 2030 tumor were trypsinized, pooled, and a suspension of cells inoculated into an adult hamster in which a rapidly progressing tumor appeared at 14 days. This hamster's serum had an HI titer of $<20$ at 27 days after receiving the cell inoculum.

*Discussion.* Ability of this polyoma virus induced fibrosarcoma to be transplanted into adult hamsters through 8 transplants establishes one additional criterion concerning its malignancy. The infrequency of development of anti-polyoma virus antibodies in those animals supporting growth of large transplanted tumors and the fact that presence of high antibody levels against the virus did not prevent a take of transplanted cells, raise questions concerning relationships between virus and tumor. Others have found that it is difficult to isolate the virus from tumors developing after infection of newborn animals but this has been explained as due to presence of high levels of antibody in serum and tumor at time of its emulsification. However, this cannot be the explanation in animals carrying transplanted tumors since no antibody was demonstrable at the time the tumor was harvested. Furthermore, on carrying the transplanted tumor through 5 tissue culture transfers over 55 days, no evidence of virus could be demonstrated. Yet virus was present in the original tumor since it was isolated from the second and third tissue culture transfer of that tumor. The late development of antibody in a few hamsters in the transplantation series suggests that virus is present in the tumors but is released very slowly and in amounts insufficient to act as an effective antigenic stimulus to the animal's immunological system.

*Summary.* A fibrosarcoma produced by inoculation of polyoma virus into a suckling hamster has been transplanted through 8 transfers in adult hamsters with preservation of its original histological character. In only an occasional animal supporting a transplanted tumor have anti-polyoma virus antibodies appeared. The transplanted tumor was carried through 5 tissue culture transfers without demonstration of virus release and tissue culture cells were capable of producing tumors in an adult hamster. After 26 days sojourn in an adult mouse, the transplanted hamster tumor still produced tumors in the hamster but was not transplantable in series in the mouse. The presence of tumors or high level of virus antibodies did not prevent a positive take with the transplantable tumor.

1. Stewart, S. E., *J. Nat. Cancer Inst.,* 1955, v15, 1391.

2. Stewart, S. E., Eddy, B. E., Gochenour, A. M., Borgese, N. G., Grubbs, G. E., *Virology,* 1957, v3, 380.

3. Eddy, B. E., Stewart, S. E., Young, R., Mider, G. B., *J. Nat. Cancer Inst.,* 1958, v20, 747.

4. Eddy, B. E., Rowe, W. P., Hartley, J. W., Stewart, S. E., Huebner, R. J., *Virology,* 1958, v6, 290.

5. Stewart, S. E., Eddy, B. E., Borgese, N., *J. Nat. Cancer Inst.,* 1958, v20, 1223.

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## Adenylic Acid and Adenosine Deaminase Activities in Rat Livers During Azo-Dye Carcinogenesis.* (25157)

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Adenylic acid and adenosine deaminases are 2 purine-metabolizing enzymes. The former catalyzes conversion of 5′-adenylic acid to 5′-inosinic acid while the latter catalyzes conversion of adenosine to inosine(1). Recently, it was shown that adenylic acid de-

* The authors express their appreciation to Bettye Cox for technical assistance.

TABLE I. Effect of 3′-ME-DAB Feeding on Adenosine and Adenylic Acid Deaminase Activities in Precancerous Livers.*

| | μM $NH_3$/mg N/15 min. | | | |
|---|---|---|---|---|
| | Adenosine deaminase | | Adenylic acid deaminase | |
| Days | Control | Dye-fed | Control | Dye-fed |
| 0 | .40 | | .46 | |
| 30 | .37 | .93 | .45 | .64 |
| 60 | .52 | 1.01 | .44 | 1.31 |
| 90 | .39 | 1.06 | .37 | .89 |

Adenosine deaminase: Adenosine, .025 M, phosphate buffer, .05 M, pH 7.0; tissue concentration, 30 mg (wet wt)/ml; 38°C.

Adenylic acid deaminase: Adenylic acid, .006 M, citric acid buffer, .05 M, pH 6.5; tissue concentration, 30 mg (wet wt)/ml; 38°C.

* Activities were determined on liver homogenates from 3 to 4 animals pooled.

aminase(2) and adenosine deaminase(3) activities in transplanted Novikoff hepatomas were considerably higher than normal liver. On the other hand, Reid and Lewin(4) found no consistent change in adenosine deaminase activity in azo dye-induced primary hepatomas. Since the transplanted Novikoff hepatoma was initially induced by an azo dye (5), it was our purpose to determine whether increases in adenosine and adenylic acid deaminase activities observed in transplanted hepatomas were the result of transplantation or occurred during azo-dye carcinogenesis. Levels of the 2 deaminases were determined in precancerous livers and in primary hepatomas induced by feeding 3′-methyl-4-dimethylaminoazobenzene (3′-Me-DAB).

*Materials and methods.* Female Holtzman rats were fed for 90 days with 3′-Me-DAB synthesized by the method of Giese, *et al.*(6) in a semi-synthetic, riboflavin-deficient diet described by Medes, *et al.*(7). Control rats received identical diet without carcinogen. Throughout the dye-feeding period, control and experimental animals were sacrificed at 30-day intervals and 3 to 4 livers were pooled for each enzymatic determination. After 90 days carcinogen was withdrawn from the diet and dye-fed animals were sacrificed individually when primary hepatomas were detected by palpation. The assay method for deaminases has been described previously(2). Tissue homogenates were incubated with either adenosine or adenylic acid and ammonia production was determined at intervals to follow extent of the reaction. Controls, in which either substrates or homogenates were omitted from the reaction mixture, were determined in each experiment and only negligible ammonia production was observed. The unit of enzymatic activity for both deaminases is expressed as μM of ammonia produced/mg homogenate nitrogen/15 minutes incubation.

*Results.* The effect of 90 day azo-dye regimen upon adenylic acid and adenosine deaminase acvtivities of precancerous livers is shown in Table I. While control livers exhibited essentially no change in deaminase activities throughout 90 days, activities of both deaminases in livers taken from dye-fed animals were elevated about 100% after 30 days and maintained this level throughout 90 days. Since pathological examinations† showed that precancerous livers were essentially free of neoplastic tissues throughout 90 days, it seemed reasonable to conclude that increases in deaminase activities had occurred prior to formation of neoplastic tissues.

When deaminase activities in primary hepatomas and liver tissues adjacent to hepatomas were determined and compared with control livers (Table II), activities in primary hepatomas and adjacent livers were considerably higher than control livers and were essentially the same as those in precancerous livers. From these data it appears that no additional change in deaminase activities occurred following formation of primary hepa-

TABLE II. Adenosine and Adenylic Acid Deaminase Activities in Primary Hepatoma and in Adjacent Liver.*

| | μM $NH_3$/mg N/15 min. | |
|---|---|---|
| | Adenosine deaminase | Adenylic acid deaminase |
| Control | .34 | .45 |
| Primary | .81 ± .02†(2)‡ | 1.33 ± .45 (5) |
| Adjacent liver | .96 ± .36 (3) | .90 ± .07 (2) |

Experimental conditions: See Table I.

* Control values from livers of 3 animals pooled; activities of primary hepatomas and adjacent livers were determined on individual animals.

† Stand. dev.

‡ No. of observations.

† The authors express appreciation to Dr. E. S. Irvine for pathological examinations.

tomas. While adenosine deaminase data in our study did not correlate with observations of Reid and Lewin(4), DeLamirande and Allard‡ reported data which, in agreement with present findings, showed increased adenosine deaminase activity in precancerous livers from 3′-Me-DAB feeding.

Comparison of enzyme activities in liver and hepatomas led Potter(8) to formulate an enzyme deletion hypothesis in which it was suggested that loss of catabolic enzymes in tumor tissues functioned to preserve metabolites for synthetic pathways associated with cell multiplication. Activities of several purine-catabolizing enzymes, such as 5′-nucleotidases, 5′-nucleoside phosphorylases, guanase and adenase, were reported(3) considerably lower in Novikoff hepatomas compared with normal liver; while xanthine oxidase and uricase appeared to be absent. Decreases of these purine-catabolizing enzymes could be interpreted as functioning to preserve purines for nucleic acid synthesis. Since adenylic acid deaminase and adenosine deaminase may function in conversion of adenine compounds to guanine compounds for incorporation into nucleic acids, their increased enzymatic potential during azo-dye carcinogenesis may be correlated with the metabolite preservation concept of Potter(8). Moreover, it is interesting to note that studies with other fast growing tissues such as chick embryos(9) and regenerating rat livers(10) also showed increased adenosine deaminase activity.

*Summary.* Adenosine and adenylic acid deaminase activities were determined in precancerous livers and in primary hepatomas induced by feeding 3′-methyl-4-dimethylaminoazobenzene. The deaminase activities in precancerous livers were increased about 100% after 30 days of dye feeding compared with control livers. Activities in primary hepatomas and in the liver adjacent to primary hepatomas were essentially the same as that of precancerous livers, indicating that no additional changes occurred upon formation of neoplastic tissues.

‡ This report was presented at Am. Assn. for Cancer Research, Atlantic City, N. J., 1959.

1. Schmidt, G., *The Nucleic Acids,* vI, Academic Press, N. Y., 1955, p596.

2. Chan, S. K., McCoy, T. A., Kizer, D. E., PROC. SOC. EXP. BIOL. AND MED., 1959, v100, 420.

3. DeLamirande, G., Allard, C., Cantero, A., *Cancer Research,* 1958, v18, 952.

4. Reid, E., Lewin, I., *Brit. J. Cancer,* 1957, v11, 494.

5. Novikoff, A. B., *Cancer Research,* 1957, v17, 1010.

6. Giese, J. E., Miller, J. A., Baumann, C. A., *ibid.,* 1945, v5, 337.

7. Medes, G., Friedmann, B., Weinhouse, S., *ibid.,* 1956, v16, 57.

8. Potter, V. R., *Fed. Proc.,* 1958, v17, 691.

9. Gordon, M. W., Roder, M., *J. Biol. Chem.,* 1953, v200, 859.

10. Thomson, J. F., Moss, E. M., PROC. SOC. EXP. BIOL. AND MED., 1955, v89, 230.

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## Determination of Apparent Hippuric Acid and Free Glycine in Urine by Microbiological Assay.* (25158)

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Values for "bound" or "combined" amino acids in urine have commonly been reported as the difference between amino acid levels found by microbiological assay of this material before and after subjecting it to acid hydrolysis (1-4). It has generally been recognized that such values may lack quantitative significance,† but that they may not be meaningful

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† Qualifying quotation marks are almost always used with the terms "free" and "bound" in this sense.