spleen or other lymphoid tissues(2,11-14) or exert any detectable effect unless carried out well in advance of transplantation.

If the foreign bone marrow reaction is similar to foreign spleen reaction in pathogenesis, as suggested by histopathologic resemblances, it could be initiated by lymphoid cells in bone marrow. In this case, differences between effectiveness of spleen and marrow would represent merely numerical differences in numbers of reactive, antibody-forming cells present in each tissue. On the other hand, the characteristically different time sequences of the 2 reactions suggest the differences between effects of marrow and spleen result from qualitative dissimilarities, yet to be elucidated. Since antibody-forming cells, not normally detectable in bone marrow, may be found there in highly immunized animals(10), the effects of pre-sensitization may conceivably represent release of such cells from spleen and lymph nodes and their migration to other sites, including the marrow.

Summary. Pre-sensitization of parental strain donor mice against F_1 hybrid recipients accentuated the foreign bone marrow reaction caused by injection of parental marrow cells into lethally irradiated F_1 recipients. This effect occurred with use of parental strains (101 and C3H) that were histocompatible at the *H-2* locus, as well as with those (C57BL and 101) that were histoincompatible at this locus. These results are interpreted to indicate the importance of relative antibody-forming potency of implanted cells, as well as the extent of histocompatibility differences in pathogenesis of the homograft reaction.

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Adenylic Acid Deaminase Activity in Spectrum of Rat Tumors and in Normal Liver.* (24648)

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Deamination of adenosine-5′-monophosphate (AMP) to inosine-5′-monophosphate (IMP) is catalyzed by the enzyme 5′-adenylic acid deaminase. This enzyme was demonstrated in skeletal muscle and in water-soluble fraction of smooth muscle, liver and kidney. Its isolation in crystalline form from rabbit skeletal muscle was recently reported. The above work was summarized by Lee(1). Greenstein *et al.*(2) observed deamination of yeast adenylic acid (presumably adenosine-3′-monophosphate) by several normal tissues of rat and mouse and by a mouse hepatoma. Recently Fodor *et al.*(3) showed that homogenates of both growing and regressing Flexner-Jobling carcinomas deaminated adenosine-5′-monophosphate, while their activity towards the 3′-isomer was much lower. Our purpose was to determine the 5′-adenylic acid deaminase activity in a number of rat transplantable tumors, and to ascertain whether activity in hepatomas resulting from dye carcinogenesis

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TABLE I. Adenylic Acid Deaminase Activity in Tumor Tissue Homogenates.

Tissues	Enzymatic activities* Homogenates		Cell suspension homogenates†	
Skeletal muscle	(1)‡	32.6		
Walker carcinosarcoma 256	(2)	7.78 ± .92§	(4)	2.78 ± .08
Jensen sarcoma	(4)	6.92 ± .80	(2)	2.47 ± .13
Flexner-Jobling carcinoma	(2)	4.38 ± .62		
Novikoff hepatoma	(7)	1.12 ± .05		

Experimental conditions described in methods section.
* Enzymatic unit: μM of ammonia produced/mg homogenate N 15 min.
† Homogenates from cell suspensions; see text, method section.
‡ No. of observations in parentheses. § Stand. dev.

was altered from that observed in normal liver.

Materials and methods. Female Holtzman rats were used. Jensen sarcoma and Walker carcinosarcoma 256 were carried intramuscularly using the transplantation method described by Talalay *et al.*(4). The Flexner-Jobling carcinoma was carried subcutaneously, while the Novikoff hepatoma and a hepatoma (MDAB) induced by feeding 3'-methyl-4-dimethylaminoazobenzene were carried intraperitoneally. At appropriate intervals the tumors were harvested and freed of gross necrotic or hemorrhagic areas. In homogenate studies the tumor tissues were stored at −25°C not exceeding 2 days. When homogenates were prepared from cell suspensions, freshly excised tumor particles were fragmented with loose-fitting glass tissue suspender in phosphate buffer (0.039 M, pH 7.4) containing 0.25 M sucrose. Cells were separated from the debris by 3 successive passages through glass-wool and filtrates were centrifuged at 100 g for 2 minutes. The supernatant was removed by suction and the cells washed once with citrate buffer (0.05 M, pH 6.5). Pathological examination of suspensions showed them to be essentially free of debris and to be contaminated with inflammatory cells to 4% by cell count.† Since deamination products of AMP were IMP and ammonia(1), ammonia production, determined by the method of Boyce(5), was used to follow extent of the reaction. Tissues or cell suspensions were homogenized in citrate buffer (0.05 M, pH 6.5) and pre-incubated 10 minutes at 38°C. Homogenates were then added to incubation mixture which consisted of 0.05 M citrate buffer (pH 6.5) and 0.006 M AMP‡ in total volume of 15 ml. Aliquots for anaysis were removed at appropriate time intervals and total incubation was 15 minutes. A linear relationship between enzymatic activity and time of incubation was obtained. Controls, in which either AMP or homogenates were omitted from the reaction mixture, were determined simultaneously in each experiment and only negligible ammonia production was observed. The unit of enzymatic activity is expressed as μM of ammonia produced/mg homogenate nitrogen/15 minutes incubation. Since inorganic phosphate concentration in the reaction mixture remained constant during the incubation period, the ammonia assayed apparently was derived directly from AMP and was not mediated by a dephosphorylation reaction.

Results. All tumor tissues studied deaminated AMP and data summarized in Table I. Homogenates of Walker and Jensen tumors exhibited highest activity among all neoplasms examined, but their activity was approximately 30% that of skeletal muscle. Since both tumors were transplanted intramuscularly, it was possible that the homogenates may have been contaminated with muscle tissues. Therefore, cell suspensions of Walker and Jensen tumors were prepared and homogenized. Under these conditions, the deaminase activity approached the level observed in Flexner-Jobling and Novikoff tumors. Thus, a portion of activity which was observed in crude homogenates of Walker and Jensen

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

‡ Pabst Labs.

TABLE II. Comparison of Adenylic Acid Deaminase Activity in Homogenates of Normal Liver, Primary Hepatoma, and Transplantable Hepatomas.

Tissue	Enzymatic activities*
Liver	(4)† .69 ± .07‡
MDAB primary hepatoma	(4) .88 ± .04
MDAB transplanted hepatoma§	(3) 3.00 ± .09
Novikoff hepatoma	(7) 1.12 ± .05

Experimental conditions described in methods section.

* Enzymatic unit: See Table I.

† No. of observations.

‡ Stand. dev.

§ This tumor has been carried for 52 transplant generations.

tumors may have been contributed by muscle or other nonneoplastic tissues.

Since Greenstein(2) reported that mouse hepatomas deaminated yeast and thymus nucleic acids to a greater extent than normal livers, the adenylic acid deaminase activity in primary and transplanted hepatomas and normal livers was compared (Table II). Activity in the 2 transplantable hepatomas (Novikoff and MDAB) was significantly higher ($P < .01$)§ than normal liver; however, activity in a primary hepatoma (MDAB primary hepatoma) was equivalent to that observed in normal liver. In other studies in which hepatoma and liver were compared(6,7), the activities of several purine-metabolizing enzymes (5'-nucleotidase, nucleoside phosphorylase, guanase, adenase, xanthine oxidase and uricase) were markedly reduced, while adenosine deaminase activity was unchanged(6) or increased(7).

§ Group comparison "t" test.

Our data suggested at least 2 explanations concerning the effect of azo dye carcinogenesis upon adenylic acid deaminase activity of liver. (a) Deaminase activity of liver was not altered during carcinogenesis but subsequent transplantation of primary hepatomas resulted in significant elevation; (b) Since it has been reported that more than one kind of tumor may be induced by azo dyes(8), the 3 hepatomas studied (Table II) may have been different neoplasms.

Summary. 5'-adenylic acid deaminase activity of a spectrum of transplantable rat tumor homogenates was determined, and all tumors studied possessed deaminase activity. Activity in the primary hepatoma induced by 3'-methyl - 4 - dimethylaminoazobenzene was equivalent to that of normal liver, while the Novikoff hepatoma and a transplanted hepatoma (MDAB) had significantly higher deaminase activity than either the primary hepatoma or normal liver.

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Serum Spheroplasts of *Shigella dysenteriae*. (24649)

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We reported(1) on isolation of strains of *Shigella dysenteriae* with inherently different resistance to the bactericidal effects of "normal" human serum and on the inverse correlation between resistance to serum and resistance to penicillin. In view of the latter relationship, which agrees with the assumption that both penicillin and serum affect synthesis and/or integrity of cell-wall components(*cf.*, 2,3), an effort was made to de-