An *in vivo* effect of nucleoprotein from Ehrlich ascites tumor cells*

The isolation of microscopically clean nuclei from Ehrlich ascites tumor cells of mice has been described by GOLDBERG, KLEIN, AND KLEIN[1]. From such nuclear preparations, reasonably good yields of nucleoprotein (NP) may be obtained by distilled water extraction. This preliminary report deals with certain observations made after intraperitoneal injections of small quantities of such highly viscous preparations into mice (C3H × 101).

In the routine transfer of the ascites tumor in this laboratory and in the controls of the experiments to be described, death of one-half the animals inoculated occurs regularly some time after the eleventh day following inoculation. Table I gives a resumé of survivors when a single small quantity of NP is injected 4 days after the ascites injection. It may readily be seen that there has been no significant change in the death rate of the treated animals that might be attributed to the NP injections.

When, however, the NP-injected controls of the experiment summarized in Table I were challenged later (35 days after receiving the single NP injection) with ascites cells, the results were strikingly different; these are summarized in Table II.

A second, more extensive, series of injections was immediately begun with another NP preparation (containing 37 μg of RNA P/ml, 154 μg of DNA P/ml, and 1.62 mg of protein/ml). This experiment is still in progress but the confirmatory results seem conclusive enough to warrant further analysis. In this group, mice which received a single 0.5 ml intraperitoneal injection of NP were challenged 13, 25, and 35 days later with ascites cells. Table III summarizes the results.

From these data it would appear that the 13-day interval is insufficient to permit the maximum effect, but that after roughly 3 weeks the results are maximal. Inasmuch as the animals of this group received less material than those of the first experiment, the failure to obtain death in all the treated animals may be interpreted to mean that the effect is dose dependent, at least in the range tried here.

It is noteworthy that except for one control animal in the first experiment no animal, experimental or control, died before the fifth day following an ascites cell injection. It should also be noted that the fifth and sixth days after the tumor challenge appear to be particularly crucial ones for the NP-injected mice. This striking "end-point" (for biological material) suggests the loss by the animal of some inherent resistance to the ascites tumor. The studies of KLEIN AND RÉVÉSZ[2] have

TABLE I

THE SURVIVAL OF MICE INJECTED WITH ASCITES TUMOR CELLS, NUCLEOPROTEIN, AND WITH ASCITES TUMOR CELLS FOLLOWED 4 DAYS LATER BY NP

Material injected	*No. of survivors in indicated no. of days after ascites injection**								
	4	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*
Ascites	9/10	9/10	8/10	7/10	7/10	7/10	7/10	4/10	3/10
Nucleoprotein (NP)	12/12	12/12	12/12	12/12	12/12	12/12	12/12	12/12	12/12
Ascites plus NP 4 days later	12/12	12/12	11/12	11/12	11/12	11/12	10/12	7/12	4/12

* The denominators are the total numbers of mice in each group; the numerators are the survivors for the day indicated.

TABLE II

THE SURVIVAL OF MICE INJECTED WITH NP 35 DAYS PRIOR TO AN ASCITES INJECTION

Material injected	*No. of survivors in indicated no. of days after ascites injection*								
	4	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*
Control ascites	11/11	11/11	11/11	11/11	11/11	8/11	7/11	7/11	7/11
NP plus ascites	12/12	3/12	0/12						

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TABLE III

THE SURVIVAL OF MICE INJECTED WITH NP AT DIFFERENT TIMES PRIOR TO AN INJECTION OF ASCITES

Interval between injections in days	*No. of survivors in indicated no. of days after ascites injection*								
	4	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*
13 NP-injected	12/12	10/12	10/12	10/12	10/12	9/12	8/12	8/12	5/12
Controls	12/12	12/12	12/12	12/12	11/12	11/12	9/12	8/12	7/12
25 NP-injected	12/12	7/12	4/12	4/12	2/12	1/12	1/12	1/12	0/12
Controls	12/12	12/12	11/12	11/12	9/12	5/12	5/12	5/12	3/12
34 NP-injected	12/12	8/12	7/12	7/12	5/12	5/12	4/12	2/12	
Controls	12/12	12/12	12/12	12/12	11/12	10/12	7/12	7/12	

shown that infiltration of solid host organs by ascites tumor cells is detectable after the fourth or fifth days following inoculation. It is conceivable that in NP-injected mice such infiltration begins somewhat earlier, and contributes to an earlier death. Quantitative studies by other workers on the Ehrlich ascites tumor have revealed an early phase of rapid growth (the *cube-root phase* of KLEIN AND RÉVÉSZ, the *exponential phase* of LUCKÉ AND BERWICK[3]) which is prominent during the second through the fifth days after injection. Therefore, it is also conceivable that the ascites cells have in some way been affected by the pretreatment of the host with NP to produce an even more rapid growth, and, hence, an earlier death to the host.

The parallelism between the effect reported here and an immune reaction is inescapable. Even though the quantity of NP was small and administered as a single dose, there is some evidence that the reaction varies directly with the dose since the NP of the second experiment is known to have been more dilute than that of the first. The necessity for an intervening period of several weeks between NP injection and the ascites inoculation to produce the premature death strongly suggests the latent period of antibody production. Finally, the recent work of BLIX, ILAND AND STACEY[4], who report the antigenicity of nucleoprotein and of deoxypentose nucleic acid from calf thymus and who show remarkable specificity in the antisera to their preparations, must be considered. If the injection of NP is producing anti-NP antisera, interpretation of the experimental results in terms of the conventional immunological reaction would be difficult; retardation rather than enhancement of tumor development would be expected. However, if the NP injections were, through some mechanism, producing a reduction in a postulated antitumor factor normally present in the mouse, the results might more readily be explained.

The extracted materials utilized in these experiments and the results obtained have a bearing on the work of at least two other recent investigations. DAY *et al.*[5] have shown an *active tissue substance* responsible for "conditioning the host" in experiments with a mouse sarcoma. In at least some of their extraction procedures nucleoproteins of the tissue utilized could have been present in such amounts as to have been administered in their multiple injections in dosages comparable to those found in the single injections used here.

KUTSKY[6] has reported the stimulating effect of nucleoproteins extracted from chick embryos on the *in vitro* development of homologous heart fibroblasts cultured on a medium supplemented with this fraction. In these experiments the nucleoprotein was associated with a relatively large quantity of ribonucleic acid and little deoxyribonucleic acid; the nucleic acids alone were shown to have no significant stimulatory effects on the tissue culture growth. In the experiments reported here special care was exercised in the extraction of the nucleoprotein to maintain such conditions as would yield a product high in undissociated deoxyribonucleoprotein; indeed, preliminary analysis has shown roughly four times more DNA than RNA. Although this product is different from that used by KUTSKY in his experiments, the possibility remains that the NP of these experiments is acting in a manner completely similar to that reported by him, namely, stimulating the ascites cells directly. Such a point of view must be based first on the assumption that the NP is retained in the animal for long periods and is utilized in some manner by the tumor cells. The lengthy literature on the use of streptokinase and streptodornase (streptococcal deoxyribonuclease) in the removal of stubbornly persistent solid elements in pleural exudates attests to the reasonableness of this assumption. Some explanation, however, must accompany this assumption in order to include the latent period apparently necessary to the production of the effect.

Present work on this problem is being directed toward (1) the better qualitative and quantitative identification of the NP preparation, (2) the specificity of the reaction, and (3) a comparison between the normal progress of ascites tumor development and its progress in NP-injected hosts. These investigations and a fuller description of the extraction procedure will be reported later.

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1 L. GOLDBERG, EVA KLEIN AND G. KLEIN, *Exp. Cell Research*, 1 (1950) 543.
2 G. KLEIN AND L. RÉVÉSZ, *J. Nat. Cancer Inst.*, 14 (1953) 229.
3 B. LUCKÉ AND MARY BERWICK, *J. Nat. Cancer Inst.*, 15 (1954) 99.
4 U. BLIX, C. N. ILAND AND M. STACEY, *Brit. J. Exp. Pathol.*, 35 (1954) 241.
5 E. D. DAY, N. KALISS, A. I. ARONSON, B. F. BRYANT, D. FRIENDLY, F. C. GABRIELSON AND PRISCILLA M. SMITH, *J. Nat. Cancer Inst.*, 15 (1954) 145.
6 R. J. KUTSKY, *Proc. Soc. Exp. Biol. Med.*, 83 (1953) 390.

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Diaminopimelic acid decarboxylase in pyridoxin-deficient *Escherichia coli*

The bacterial amino acid, diaminopimelic acid (DAP) is decarboxylated to L-lysine by a specific enzyme (DAP decarboxylase) which occurs in many bacteria[1,2,3,4]. This enzyme differs from the majority of the bacterial amino acid decarboxylases in that it is a constitutive enzyme functioning at physiological pH, but it resembles them in its activation by pyridoxal phosphate. In *Escherichia coli*, the function of DAP decarboxylase is probably to provide a biosynthetic route to lysine[5].

Using a pyridoxin-requiring mutant of *E. coli* (derived from 154-59L), a study has been made of the effects of pyridoxin deficiency on the cellular levels of DAP decarboxylase and also of the adaptive lysine decarboxylase. Pyridoxin deficiency was produced by growing the mutant on a simple medium (DAVIS AND MINGIOLI[6] with 0.5% glucose and 0.45% citrate) containing the minimal level of pyridoxin (20 μg/l) necessary for growth. Control cells were grown on a pyridoxin level of 500 μg/l. These media were used both with and without supplements of L-lysine (200 mg/l). A 24 h culture in 100 ml of medium containing 2 μg pyridoxin was used as an inoculum for 2 l batches of medium; growth was continued for 24 h at 37° in the dark with vigorous aeration. The cells were harvested by centrifuging, washed and acetone-dried. DAP and lysine decarboxylases were measured at pH 6.8 in the Warburg apparatus both with and without added pyridoxal phosphate. pH 6.8 was chosen as being near the optimum for DAP decarboxylase in *E. coli*[1]; this is not the optimum for lysine decarboxylase, the values for which were about twice as high at pH 5.0 as at pH 6.8. Since lysine decarboxylase, when present, is operative in the measurement of DAP decarboxylase[3], the values for both enzymes are reported at the same pH.

Vitamin B_6 assays were carried out microbiologically by a method modified somewhat from that of ATKIN *et al.*[7]. The test organism was a variant of *Saccharomyces carlsbergensis* 4228 which required pyridoxin absolutely for growth even in the absence of thiamine. The basal medium contained less casein hydrolysate (0.2% final) and the cultures were sloped (not shaken) during a 22 h incubation. The samples were extracted with 45 ml 0.055 *N* H_2SO_4 at 121° for 1 h before assaying the supernatant. The method assays equally all known forms of vitamin B_6 and the results are expressed in terms of pyridoxin.

The results (see Table) show that the cells grown on minimal pyridoxin contained only about 11% of the pyridoxin of the control cells. The presence of lysine in the growth medium apparently had a pyridoxin-sparing effect, since in both deficient and control cells the lysine-grown cells had a higher pyridoxin content. The possibility of contamination of the lysine by traces of pyridoxin was not investigated, but it seemed unlikely in view of the similarity of the results obtained with high and low pyridoxin levels. Addition of pyridoxal phosphate to the Warburg flasks caused stimulation of decarboxylase activity in pyridoxin-deficient cells, but had little effect on the control cells, showing that a genuine state of pyridoxin deficiency had been produced. Lysine decarboxylase was absent from both cultures grown without additional lysine. The apoenzyme of DAP decarboxylase (estimated in the presence of pyridoxal phosphate) was about twice as high in the deficient cells grown without lysine as it was in the control cells or in the deficient cells grown with lysine. On the other hand, the coenzyme-bound DAP decarboxylase (holoenzyme, estimated without additional pyridoxal phosphate) was markedly reduced in the deficient cells grown in the presence of lysine.