in some fashion so that the level of fecal thiamine is increased. The antibiotic may eliminate certain strains of microorganisms with those surviving being more proficient producers of thiamine. Antibiotics also show a comparable effect in riboflavin and pyridoxine deficiencies (5).

One might propose a similar mode of action for dieldrin, but this would also imply that this chemical is exerting some selective effect on the intestinal microflora. A toxic chemical such as dieldrin may well have some bactericidal effect, but there is no evidence to suggest that its action is particularly selective. Also, in some preliminary experiments, dieldrin has not shown any sparing effect in riboflavin and pyridoxine deficiencies. A proposed experiment with axenic rats should throw some light on this situation.

Another possibility could be that dieldrin improves the efficiency of thiamine utilization. This could be effected by stimulation of absorption or by inhibition of metabolic breakdown which would decrease turnover rate and conserve tissue levels of the nutrient.

These data also illustrate a genetic effect. There is a marked variation among litters in their response to the low thiamine ration and the degree of the dieldrin response is greater in those litters which are more susceptible to the thiamine stress. If one assumes that the variation in response to the nutritional stress is genetic in nature, this interrelation might be explained on the basis of an effect of dieldrin on the intestinal production of thiamine. If the onset of the nutritional deficiency promotes coprophagy those animals more subject to the stress could benefit more from an increase in the level of fecal thiamine.

Summary. A dietary level of 20 ppm of dieldrin increased growth in rats raised on rations containing low levels of thiamine, or conversely, the low dietary intake of thiamine produced a smaller growth depression in rats ingesting dieldrin. There was a marked variation among litters in their response to the nutritional stress and the dieldrin produced a more pronounced response in those litters which were more susceptible to the thiamine deficiency stress.

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The Inhibition of the Maturation of Newly Synthesized Bone Collagen by β-Aminopropionitrile in Tissue Culture* (33345)

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One of the principal manifestations of lathyrism in animals is the increased solubility of tissue collagens, or more precisely, the increased ease with which collagen can be extracted from tissues and the increased quanti-

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ty of tissue collagen which can be dissolved (1–3). This is particularly true of bone collagen, which in normal animals is only very sparingly soluble in the solvents usually used to extract the undenatured protein (1,2). Although the lathyritic collagen macromolecules have been shown to be essentially normal in shape, size, conformation, and composition (1,4), a striking decrease in the number of covalent crosslinkages between the alpha chains has been demonstrated, especially in the acid-soluble extracts (5,6). More recent evidence suggests that this may be due, in part at least, to a decrease in the aldehyde content of the α-chains, more specifically an absence of an aldehyde function on one of the lysine residues (7,8). The absence of this aldehyde component may also play an important role in the intermolecular interaction properties of collagen macromolecules (9), which are also markedly altered in this disease (4). However, although lathyrism probably affects both the chemical composition (aldehyde formation) and the interaction properties of tissue collagen, it is still not clear whether the lathyrogenic agents only inhibit the formation of such crosslinkages (3,10–13) or whether they also cleave crosslinkages formed prior to the induction of the disease (1,14,15).

In order to investigate this problem, the effect of β-aminopropionitrile (βAPN) on the solubility properties of bone collagen was studied in tissue culture, since it has previously been shown that lathyrogenic agents can produce the characteristic abnormalities of lathyrism in bone in tissue culture (10,11,16).

Experimental Methods. The frontal and parietal portion of the calvaria of 5-day-old Swiss albino mice were dissected free and incubated in roller-tube cultures (17,18). Three different tissue culture systems were used. In the first, active bone collagen formation and resorption both occur (RF system) (17); in the second, bone collagen is not synthesized but bone collagen is actively resorbed (R system) (17,18); in the third system (10% O_2) there is both minimal bone collagen formation and resorption (17).

In the first group of experiments employing the RF or remodeling system, individual calvaria were incubated in 2-ml aliquots of a stock solution containing proline-^{3}H (0.6 μCi/ml), with and without the addition of βAPN at a final concentration of 100 μg/ml. The calvaria were incubated for a total of 10 days, the media being changed at 2-day intervals. At the end of the experimental period the calvaria were washed briefly, and the soluble bone collagen was extracted in 3–5% acetic acid at 4° for 3 days (2). Further demineralization of the calvaria in 0.5 *M* EDTA, pH 8.3, at 4° for 5 days, followed by further extractions in 3% acetic acid for 48 hr each, removed no further collagen. The insoluble residue of the calvaria and the solubilized collagen were hydrolyzed in 6 *N* HCl at 105° for 24 hr and the hydroxyproline was separated from the proline by chromatography on Dowex 50, H^+-resin (17). Hydroxyproline was determined colormetrically (19) and hydroxyproline-^{3}H was measured in an automatic liquid scintillation counter. Similar procedures were used in the R system except that the media contained no proline-^{3}H.

In a second group of experiments, calvaria were incubated in the RF system in the presence of proline-^{3}H for 6 days, rinsed gently, and then incubated for 2 days in a nonradioactive RF system. The calvaria were then reincubated for a final 6-day period in a nonradioactive RF system with and without added βAPN (100 μg/ml) either in the presence of 20% O_2 or in 10% O_2. At the end of this 14-day period the soluble and insoluble hydroxyproline-^{3}H content of the calvaria was determined.

In a third group of experiments, freshly dissected calvaria were killed by drying at room temperature for 7 days and then were allowed to incubate in the RF system containing proline-^{3}H, with or without 100 μg of βAPN/ml for 10 days. After this incubation, the calvaria were analyzed as described for the first group of experiments in which living calvaria were similarly incubated.

Results. In the RF tissue culture system, in which both the synthesis and degradation of bone collagen occur, the addition of βAPN to the tissue culture medium resulted in a marked increase in the amount of collagen

TABLE I. The Effect of βAPN on the Solubility of Bone Collagen in Calvaria after Incubation in Tissue Culture (10 days).[a]

βAPN (μg/ml)	Type of incubation system	Hydroxyproline solubilized: % of total hydroxyproline content of tissue	Hydroxyproline solubilized: % of hydroxyproline-^{3}H content of tissue
None	RF	4	3
100	RF	24	79
None	R	4	—
100	R	4	—

[a] Each value is the mean of 2–4 samples.

that could be extracted from the tissue (Table I). Moreover, 79% of the collagen newly synthesized in tissue culture in the presence of βAPN was solubilized. On the other hand, when similar concentrations of βAPN were added to the tissue culture media of the R system, in which new collagen synthesis does not occur, but in which bone resorption does occur, no increase in the amount of soluble bone collagen was detected (Table I).

The effect of βAPN on normal bone collagen synthesized in tissue culture prior to exposure to this agent is shown in Table II. These data clearly show that essentially none of the hydroxyproline-^{3}H-labeled collagen synthesized prior to incubation with βAPN is sufficiently altered by 6 days of exposure to βAPN to render it soluble (in another experiment, βAPN was found to markedly increase the solubility of collagen synthesized in its presence after 6 days in tissue culture). Although practically no hydroxyproline-^{3}H could be detected in the soluble collagen, it is clear that collagen was synthesized in tissue culture as demonstrated by the finding of approximately 800 cpm hydroxyproline-^{3}H in the insoluble collagen of the calvaria incubated in the 10% O_2 system and about 5500 cpm in the insoluble collagen of calvaria incubated in the presence of 20% O_2.

Further evidence that bone collagen synthesized prior to exposure to βAPN is not affected, was the finding that bone collagen of devitalized calvaria incubated in the RF system was not rendered soluble after 10 days of exposure to βAPN. No collagen was synthesized during the incubation as demonstrated by the failure to detect any conversion of proline-^{3}H to hydroxyproline-^{3}H.

Discussion. Martin and Goldhaber (1963) demonstrated that the lathyrogenic agent, aminoacetonitrile (AAN), could produce the same effect on collagen solubility in tissue culture as had previously been observed to occur *in vivo* (20). These results obtained from tissue culture experiments have been confirmed in the present study using βAPN as the lathyrogen. While these solubility data provide evidence that AAN and βAPN act directly on bone tissue and increase the solubility of bone collagen, they do not distinguish whether the lathyrogens affect the newly synthesized collagen by inhibiting the formation of crosslinks or whether they act on collagen synthesized prior to the exposure

TABLE II. The Effect of βAPN on the Solubility of Bone Collagen Synthesized Prior to Exposure to βAPN.[a]

Incubation procedure in the RF system				
A. Incubation in media containing proline-^{3}H (days)	B. Subsequent incubation, 6 days, in media containing no proline-^{3}H (% O_2)	βAPN added to the subsequent incubation (B) (μg/ml)	Soluble collagen (% of the total hydroxyproline-^{3}H content of tissue)	Insoluble collagen [hydroxyproline-^{3}H remaining in the residue after extraction (cpm)]
6	20	None	<1	5889
6	20	100	<1	5160
6	10	None	0	778
6	10	100	0	756

[a] Each value is the mean of 4 samples.

to the lathyrogen by disrupting crosslinks already formed.

Fessler and Bailey (1966) found that βAPN cleaved the intramolecular covalent crosslinkages of collagen *in vitro*, converting the β-dimers to the single stranded α-chains. These results, which indicate that βAPN is capable of altering already formed collagen macromolecules, suggest that the effects of βAPN noted *in vivo* may be due, in part, to the cleavage of crosslinkages synthesized prior to the exposure to the drug. On the other hand, Martin *et al.* 1963), studying the incorporation of glycine-^{14}C into the subunits of collagen from normal and lathyritic animals, concluded that the major effect of the lathyrogen was on that fraction of the tissue collagen synthesized during exposure to the drug, but these authors were unable to rule out the possibility that previously synthesized collagen was also affected to some degree. In other experiments, Martin *et al.* (1966) reported that it was the bone collagen synthesized in tissue culture in the presence of βAPN which was primarily affected. However, approximately 15% of the hydroxyproline-^{14}C-labeled collagen synthesized prior to the exposure to βAPN was solubilized after exposure to βAPN (as compared to 1% in the control samples), and it was not possible to distinguish whether this represented collagen synthesized prior to the addition of βAPN to the media, or whether it represented collagen synthesized during the exposure to the βAPN. This is due to the fact that when bone is incubated in tissue culture, bone resorption as well as bone formation may occur. The resorption of bone collagen in tissue culture has been found to result in the release into the tissue culture media of radioactive and unlabeled proline as the free amino acid (21) which could then be incorporated into the collagen which is being synthesized in the presence of βAPN. The experiments reported in this paper were designed to overcome this difficulty. Bone collagen was synthesized in tissue culture in the presence of proline-^{3}H. This permitted us to identify bone collagen synthesized prior to the exposure to βAPN, since it was labeled with hydroxyproline-^{3}H. These bone samples were then reincubated in tissue culture with βAPN under conditions in which minimal bone resorption and formation occurred, so that there was little likelihood of a significant reincorporation of proline-^{3}H into collagen synthesized in the presence of βAPN. Under these conditions, no increase in the solubility of the labeled bone collagen, representing protein synthesized in the absence of βAPN, was noted.

The following additional data demonstrated that it is only the collagen synthesized in the presence of βAPN which is affected by the lathyrogen and not the collagen formed prior to the introduction of this agent. The βAPN was found to have no effect on the solubility of preformed collagen present either in devitalized calvaria incubated in a remodeling type (RF) of tissue culture system in which collagen synthesis can occur, or in vital calvaria incubated in a resorbing system (R) in which collagen synthesis does not occur.

These data clearly demonstrate that βAPN does not affect collagen already present in bone tissue but acts only on that collagen which is synthesized in the presence of the lathyrogen.

Summary. The effect of βAPN on bone collagen was studied in tissue culture. It was found that the lathryrogen modified the solubility characteristics of only that fraction of collagen which was synthesized in the presence of βAPN, and did not affect the bone collagen which was synthesized prior to the exposure of the tissue to βAPN.

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Partial Purification of Chromosomal DNA Polymerase from Rat Walker Tumor* (33346)

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Previous studies in this laboratory have shown that the nonhistone chromatin proteins from rat liver and calf thymus contain a replicative type DNA polymerase (1, 2). It was subsequently found that isolated cell nuclei from solid Walker tumor are especially rich in this enzymic activity. Investigation of the acidic chromosomal proteins from Walker 256 carcinosarcoma has resulted in the partial purification of a DNA polymerase. The method of the enzyme purification is described in the present report. It will be shown that the purified DNA polymerase requires only native DNA as template and a complete supplement of all four deoxyriboside triphosphates as substrates. The enzymic reaction also depends on the presence of Mg^{2+}, monovalent cations being inhibitory.

Materials and Methods. Walker 256 carcinosarcomas were developed for 7 days after tumor cells were injected intramuscularly into the hind legs of rats. The tumor cells had a mitotic cycle of 22–25 days and showed well-organized endoplasmic reticulum network. All the following operations to be described were carried out at 2–4°.

Cell nuclei were isolated from the tumor tissue by the method of Chauveau *et al.* (3). The tumor nuclei were extracted with 100 vol of 0.05 *M* Tris–HCl, pH 7.4, containing 5 m*M* $MgCl_2$, followed by repeating the buffer extraction three times. The nonhistone chromosomal proteins were prepared from the washed nuclei according to previously described procedure (4).

The isolated acidic chromosomal proteins were dialyzed overnight against 0.05 *M* Tris–HCl, pH 8.5, containing 1 m*M* 2-mercaptoethanol. To the dialyzed acidic protein solution, saturated ammonium sulfate (AS) solution (adjusted to pH 8.5 with NH_4OH) was added slowly with stirring to 40% saturation with respect to ammonium sulfate. After standing for 15 min, the mixture was centrifuged at 10,000*g* for 15 min and the precipitate was discarded. To the clear supernate enough ammonium sulfate was added to a final 60% saturation of ammonium sulfate. The precipitate (40–60% AS) thus formed

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