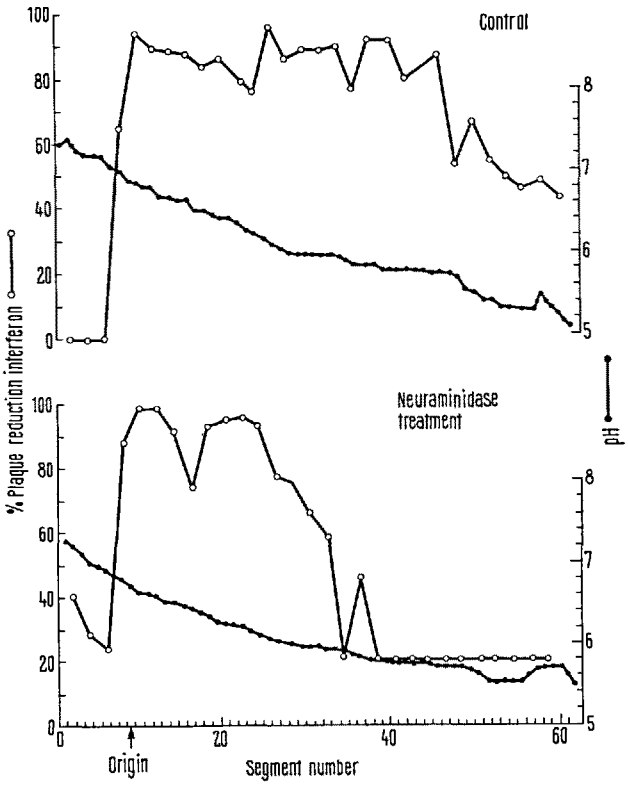
By using a short incubation period, it has been possible to obtain almost complete desialization while preserving 94% of the original interferon activity.

Polyacrylamide gel was prepared by the polymerization of 6% (w/v) acrylamide containing 2% (w/v) 'Ampholine' carrier ampholytes (pH 5–8). Polymerization of the gel was induced by riboflavine. After loading the samples (neuraminidase-treated and control urinary proteins containing interferon), the surface of the gel was covered with petroleum jelly and isoelectric-focusing[8] was carried out in the vertical position: the cathode was wetted with 5% (v/v) ethylenediamine solution and the anode with 5% (v/v) phosphoric acid. A dialysis membrane prevented liquid flow from the electrode vessels to the gel. The run was carried out in the cold room for 30 h with a potential difference of 9 V/cm.

At the end of the run, the gel was divided[9] into 61 segments (0.3 cm) for the neuraminidase-treated and control samples, respectively. In order to measure the pH, $^1/_3$ of the segment was suspended in 4.5 ml of distilled water; in order to recover the fractionated interferon, the remaining segment was immersed into 5 ml of medium 199 containing 20% bovine serum and was left shaking for 60 h at 0°.



Simultaneous separation of neuraminidase-treated and control urinary proteins containing interferon by polyacrylamide – isoelectric – focusing in a pH 5–8 ampholyte system.

The Figure shows the result of a typical run: the control urinary interferon consists of several active components (maybe 7 or more) of different isoelectric point ranging from 6.9 to 5.5. After desialyzation the electrophoretic pattern is strikingly changed and shows 2 main peaks having isoelectric points at pH 6.6 and 6.3 while most of the more acidic interferon has disappeared. The heterogeneity of the interferon seems therefore due, at least in part, to the presence, possibly in a different amount, of sialic acid in the molecule.

The result suggests strongly that interferon is a glycoprotein and that removal of sialic acid raises the isoelectric point and reduces the electrophoretic mobility. The reason(s) of interferon heterogeneity remains as yet conjectural; in fact it could be due to difference in sialic acid addition at the site of synthesis, as it could be due to partial desialyzation during transit in the body or during purification procedures.

While this manuscript was in preparation a report by SCHONNE et al.[10] on isoelectric focusing of rabbit interferon has appeared. Although the source of interferon was different, the same conclusion has been reached in both studies[11].

Riassunto. Proteine urinarie di coniglio contenenti interferone sono state desializzate mediante neuraminidasi e sono state separate simultaneamente ai controlli mediante elettroforesi su poliacrilammide con gradiente di pH. La rimozione dell'acido sialico produce una notevole modificazione del profilo elettroforetico con diminuzione dell'attività interferonica avente punti isoelettrici inferiori a pH 6.3. Il risultato indica che almeno in parte l'interferone contiene acido sialico.

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Experimental Lathyrism: Inhibition of β-Alanine Incorporation by β-Aminopropionitrile

The nature of the defect in experimental lathyrism has been extensively reviewed by other investigators[1–3]. Essentially, it is a disease of connective tissues exhibiting changes in collagen, elastin, and ground substance.

The nature of the defect in collagen has been shown to be an increase in the amount of the neutral salt soluble fraction, and the failure of collagen molecules to mature into normal fibrils. This defect has been attributed to modification in the cross-linking between collagen molecules occurring at both the inter and intramolecular level[4,5]. Changes in elastin are also thought to be due to a failure in cross-linking. Whether this is accompanied by a decreased synthesis of elastic fibers or a destruction and fragmentation of those fibers already produced is not clear[6,7]. Changes in the ground substance have been attributed principally to alterations in mucopolysaccharides.

The mechanisms by which lathyrogens produce the disease is still unknown. Nitriles are said to be amine reactors under physiological conditions and may be expected to enter into peptide linkages in the synthesis of proteins[8]. The finding of β-alanine, a possible hydrolytic product of β-aminopropionitrile (BAPN), in the total hydrolysate of lathyritic collagen supports the contention that lathyrogens can be involved and act directly with a protein such as collagen[9]. What is more intriguing is the possibility that because of the similarity between a lathyrogen such as β-aminopropionitrile and the amino acid β-alanine, lathyrogens may act as antimetabolites competing with a structurally related metabolite for incorporation into developing connective tissues[10]. The purpose of this investigation was to test this latter hypothesis using an in vitro model system previously described[11].

Materials and methods. L-929 fibroblasts were grown in EAGLE's[12] minimum essential medium (MEM) supplemented with 10% calf serum and grown in an atmosphere of 5% CO_2 and air. A cell suspension having a population of 1×10^5 cells per ml was prepared from 5-day-old cultures. C^{14} labeled β-alanine (carboxyl carbon labeled) was then added to the cell suspension, 2 μC per ml of culture medium used. The cell suspension was divided into 5 parts; 1 served as a control, and BAPN was added to the other 4 giving the following m*M* concentrations: 10, 5, 3, and 1. Replicate cultures of each concentration were prepared using 60×15 mm plastic petri dishes. Cells of each concentration were harvested at 1, 2, 3, 4, and 7 days. At harvest, cells were washed 3 times with phosphate buffered saline and dissolved in 10% sodium hydroxide. Cellular uptake of β-alanine was measured in a Beckman LS-150 liquid scintillation counter.

Results. The Table shows a gradual inhibition of β-alanine incorporation as the concentration of BAPN was increased. Viability of the cells was not affected even at the highest concentration of the lathyrogen. β-alanine is not included in the chemical formulation of MEM, but traces may be present in the calf serum added. In spite of this, the data gives measurable evidence of inhibition by the lathyrogen of cellular incorporation of the labeled β-alanine.

A concentration of 5 m*M* of BAPN has been found to depress proliferation of cells in culture[11]. Since the effect of an antagonist can often be reversed by simultaneous administration of the normal metabolite, a series of experiments were performed using concentrations of unlabeled β-alanine from 0.1 m*M* to 10 m*M* in a culture system having a 5 m*M* concentration of BAPN. None of the concentrations of β-alanine had any effect in reversing the effect of BAPN on cell growth. Although it was not the purpose of this study to determine the fate of the incorporated β-alanine, it was found to be in a non-dialyzable moiety and not precipitated by 5% trichloroacetic acid.

Discussion. The inhibition of β-alanine incorporation by BAPN suggests that there exists a degree of competition between the two; however, the effect of the lathyrogen was not truly proportional to the β-alanine over a wide range of concentrations. Although the inhibition was not truly competitive in the classical sense, one must conclude from the data that competition does exist. Not all antimetabolites are effective antagonists in all situations, but they do in general inhibit the metabolism of the normal analog. Conversely, the failure of β-alanine to reverse the effect of BAPN again demonstrates the unpredictability of antagonists since there are instances where they are less reversible or irresversible.

From the data presented in this study, it is possible that lathyrogens may act as antimetabolites competing with a normal analog for incorporation into developing connective tissues at one or more stages of metabolism. The effect on total metabolism depends upon the fit of the analog to the active site of a particular enzyme. If such an analog competes with an amino acid and is accepted by the active site of an enzyme it may function during the enzymatic reactions involved in protein synthesis and be incorporated in protein, but its presence in the protein may alter the function of the final product significantly. In this way, antagonists may play important roles in the etiology of disease by altering cellular concentrations of one or more normal analogs producing altered end products which may result in abnormal function and disease.

Zusammenfassung. β-Aminopropionitrile verhindern wirkungsvoll die zelluläre Einlagerung von β-Alaninen (strukturmässig zu den Aminosäuren gehörend) in grosser Konzentrationsbreite. Aus diesem Grunde wirken Lathyrogene als Antagonisten durch Veränderung der zellulären Konzentration der Analogen. Die veränderten Produkte haben wahrscheinlich eine abnorme Funktion und die Erkrankung zur Folge.

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Cellular incorporation of C^{14} labeled β-alanine in cells treated with a range of concentrations of β-aminopropionitrile (BAPN)

Time (days)	Control	β-aminopropionitrile treated cells (m*M*)			
		1	3	5	10
1	429[a]	427	372	375	337
2	13,123	10,361	8,173	4,294	592
3	13,988	11,358	6,965	3,283	1,429
4	28,211	27,358	18,952	12,870	2,393
7	42,898	22,396	10,607	6,360	2,650

[a] Each figure is the average of 3 samples and is expressed in cpm per 1×10^6 cells.

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