Experimental Lathyrism: in vitro Model Systems

Experimental lathyrism is a connective tissue disease exhibiting changes in collagen, elastin and ground substance[1-3]. The nature of the defect in collagen has been shown to be a failure in cross linking which in turn is manifested by an increase in neutral salt soluble collagen in lathyritic animals[4-6]. The effect of lathyrogens on the ground substance, more specifically the polysaccharides found therein, has been conflicting with reports in the literature of no change, an increase, and decrease in total synthesis[7-10].

It has been suggested and supported that the fibroblast is involved in the formation and secretion of connective tissue constituents[11,12]. Since the basic defect in experimental lathyrism appears to involve almost exclusively connective tissues, the use of lathyrogen-treated fibroblasts, in culture, should contribute not only to a better understanding of the mechanisms and pathogenesis of this disease, but also to a better understanding of the dynamic processes involved in cellular proliferation, metabolism, and possibly differentiation, in general. Although several investigators[13-15] have used β-aminopropionitrile treated fibroblasts in culture, the development of model systems using other known lathyrogens has not been reported.

The success of any in vitro model system using drugs such as lathyrogens depends upon the establishment of a dosage which will have a maximum effect upon the tissue being studied without exhibiting any toxic manifestations. One of the simplest parameters to investigate is cell growth using a hemocytometer chamber or an electronic particle counter for cell counts. In order to assess whether changes in cell growth are manifestations of drug toxicity, vital staining methods must also be employed. Vital staining methods using neutral red, Janus green B, lithium carmine, or dye exclusion tests using trypan blue, for example, are standard methods. Perhaps more valuable criteria are the investigation of generation time in the logarithmic growth phase, the ability of the cells to settle out of suspension, adhere to a glass surface and assume the usual spindle shape.

Material and methods. The model systems developed in this study utilized the following criteria: (1) proliferation of cells for a 96 h period, (2) viability counts using trypan blue exclusion, (3) morphologic studies using phase contrast microscopy, and (4) the adherence of cells to a glass surface.

Optimum concentrations for experimental study were determined for eleven known lathyrogens. Sequential concentrations of each lathyrogen were used on replicate cultures of Strain L and 3T6 mouse fibroblasts. A control was used for each lathyrogen by using the equivalent salt for each compound. In cases where this was not possible, phosphate buffered saline was used. L cells were grown in Eagle's Minimum Essential Medium and 3T6 cells were grown in the Dulbecco-Vogt modification of Eagle's medium. Both media were supplemented with 10% calf serum and grown in an atmosphere of 5% CO_2 and air.

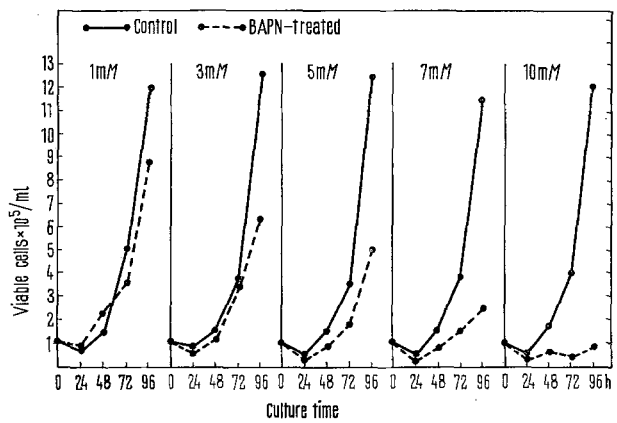
Results. Figure 1 is an example of the proliferation data of the 3T6 fibroblast using various concentrations of one of the lathyrogens, β-aminopropionitrile fumarate (BAPN). Control cultures received an equivalent concentration of sodium fumarate. Although growth was depressed with as little as a 1 mM concentration of BAPN, it took a 7 mM concentration to affect viability and the other criteria listed above. The Table lists the optimum concentrations determined for all the lathyrogens tested. Note that the concentrations vary from 0.1 mM to 5 mM. The results with the Strain L cell were identical with those of 3T6 fibroblast.

Discussion. The development of these model systems should stimulate tissue culture research in experimental lathyrism. Although the approach has its limitations, the

Optimum concentration of all the lathyrogens tested

Lathyrogen	Concentration (mM)
β-aminopropionitrile fumarate	5.0
Aminoacetonitrile bisulfate	3.0
Methyleneaminoacetonitrile	0.8
Acetone semicarbazone	1.0
Semicarbazide hydrochloride	1.0
Isonicotinic acid hydrazide	3.0
Benzohydrazide	3.0
Thiosemicarbazide	0.5
Cyanoacetic acid hydrozide	1.0
Hydrazine hydrate	0.1
1,1, Dimethylhydrazine	1.0

Note that they vary from 0.1 mM to 5 mM



The growth curves of BAPN treated 3T6 fibroblasts are shown at the various concentrations tested. Although growth was depressed, viability of BAPN treated cells did not become apparent until 7 mM of the lathyrogen was used.

1 H. Selye, Rev. canad. Biol. *16*, 1 (1957).
2 A. L. Weaver, Arthritis and Rheum. *10*, 470 (1967).
3 A. F. Gardner, Am. J. clin. Nutr. *7*, 213 (1959).
4 J. V. Hurley and K. N. Ham, J. expl Path. *40*, 216 (1959).
5 C. I. Levene and J. Gross, J. expl Med. *110*, 771 (1959).
6 G. R. Martin, J. Gross and K. A. Piez, Biochim. biophys. Acta *53*, 599 (1961).
7 D. W. Churchill, S. Gelfant, J. J. Lalich and D. M. Angevine, Lab. Invest. *4*, 1 (1955).
8 C. J. Schwartz, Br. J. expl Path. *40*, 41 (1959).
9 D. G. Walker and Z. T. Wirtschafter, A.M.A. Archs Path. *61*, 125 (1956).
10 Z. T. Wirtschafter, A.M.A. Archs Path. *64*, 577 (1957).
11 A. H. T. Robb-Smith, in *Connective Tissue in Health and Disease* (Ed. G. Asboe-Hansen; Munksgaard, Copenhagen 1964), p. 15.
12 R. C. Curran and J. S. Kennedy, Nature *175*, 435 (1955).
13 H. C. Bickley and J. L. Orbison, Lab. Invest. *13*, 172 (1964).
14 N. J. Burzynski, Lab. Invest. *12*, 816 (1963).
15 J. J. Aleo, J. L. Orbison and W. B. Hawkins, Lab. Invest. *17*, 425 (1967).

results obtained may provide leads for further and more intensive in vivo study. Whether the information gained in the study of isolated cell systems can be extrapolated to the intact animal is an area for speculation. The method does, however, offer some insight into complex processes at a cellular level which would otherwise be impossible to study in the intact animal. Also established was the difficulty to find any relationship between the structural formulas of these compounds and the concentration needed to produce toxic effects. While the lathyrogens exhibit a common effect in the animal, it is doubtful that their mechanisms of action are necessarily similar[16].

Zusammenfassung. Für 11 bekannte lathyrogenische Substanzen wurden In-vitro-Modellsysteme entwickelt. Die günstigste Dosierung entsprach der höchsten atoxischen Konzentration des Lathyrogens.

J. J. ALEO

Temple University, School of Dentistry, Department of Pathology, Philadelphia (Pennsylvania 19140, USA), 18 February 1969.

[16] Supported by the National Institutes of Health, United States Public Health Grant No. DE-AM-02547.

The Effect of Imipramine and Desipramine on UDP-Glucuronyltransferase

Imipramine (N-(3-dimethylaminopropyl) imino-dibenzyl hydrochloride) and desipramine (N-(3-methylaminopropyl) iminodibenzyl hydrochloride) are iminodibenzyl derivatives used extensively as antidepressants[1]. After imipramine administration relatively high concentrations are found in the liver, brain and lungs, the distribution being similar to the phenothiazines[2]. The major pathways of imipramine metabolism involve hydroxylation[3] and N-demethylation as well as glucuronide conjugation. Desipramine is formed by the demethylation of imipramine and has been considered to be the pharmacologically active form of imipramine[4].

Jaundice has been recorded in 0.5% to 1% of patients treated with imipramine[1]. The jaundice is transient and clears promptly when administration of the drug is discontinued[5]. The jaundice is usually cholestatic in type and resembles jaundice due to phenothiazines although a fatal case of hepatic necrosis associated with combined imipramine and desipramine therapy has been described[6]. Since chlorpromazine and some related drugs inhibit UDP-glucuronyltransferase (UDP-glucuronate glucuronyltransferase (acceptor unspecific) E.C.2.4.1.17.) we have examined the effect of imipramine and desipramine on conjugation mechanisms in rat and guinea-pig liver using in vitro systems in order to determine the possible cause of the jaundice. Bilirubin conjugation was determined in rat liver slices and guinea-pig liver homogenates by the method of LATHE and WALKER[7]. *o*-Aminophenol conjugation was determined in rat liver slices by the method of LEVVY and STOREY[8] and in guinea-pig liver homogenates by the method of STEVENSON and DUTTON[9].

The addition of imipramine and desipramine lowered the rates of conjugation of *o*-aminophenol by rat liver slices. Imipramine decreased the rate of bilirubin conjugation but desipramine did not consistently decrease the rate of bilirubin conjugation by rat liver slices (Table I). The transferase stage of conjugation was examined in guinea-pig liver homogenates incubated with ample uridine diphosphate glucuronic acid. Imipramine reduced the rates of conjugation of bilirubin and *o*-aminophenol but desipramine only reduced the rate of conjugation of *o*-aminophenol (Table II).

The effect of imipramine and desipramine on rat liver slices was investigated further by determining the conjugated bilirubin in the liver slice after 2 h incubation[10]. Imipramine decreased the amount of conjugated bilirubin in the slice whereas desipramine did not (Table III). Both imipramine and desipramine increased the unconjugated bilirubin in the liver slice.

Jaundice following imipramine and desipramine therapy is usually cholestatic in type but they can also cause hepatocellular necrosis. The jaundice is similar to that seen after phenothiazine administration. Imipramine and desipramine resemble the phenothiazines since they are metabolized in liver microsomes to form hydroxylated and desmethyl metabolites which are excreted unchanged or conjugated with glucuronic acid. Imipramine and desipramine inhibit *o*-aminophenol conjugation in liver slice and homogenate preparations. Imipramine inhibits bilirubin conjugation in rat liver slices and guinea-pig homogenates but desipramine does not consistently inhibit bilirubin conjugation. This could be explained

Table I. Effect of imipramine and desipramine on conjugation in rat liver slices

Concentration (m*M*)	Rate of conjugation (nmoles/mg wet wt./h) Imipramine		Desipramine	
	Bilirubin	*o*-Aminophenol	Bilirubin	*o*-Aminophenol
0	0.055	0.36	0.041	0.36
0.01	0.047	0.34	0.023	0.28
0.1	0.030	0.27	0.017	0.23
1.0	0.016	0.07	0.023	0.07
10.0	0	0.05	0.019	0.04
	(6)	(6)	(5)	(3)

Number of experiments in parentheses.

[1] G. L. KLERMAN and J. O. COLE, Pharm. Rev. *17*, 101 (1965).
[2] J. V. DINGELL, W. A. M. DUNCAN and J. R. GILLETTE, Fedn Proc. *20*, 173 (1961).
[3] J. R. GILLETTE, Fortschr. Arzneimittel-Forsch. *6*, 11 (1963).
[4] F. SULSER, J. WATTS and B. B. BRODIE, Ann. N.Y. Acad. Sci. *96*, 279 (1962).
[5] P. C. S. HOAKEN, Can. med. Ass. J. *90*, 1367 (1964).
[6] W. J. POWELL JR., J. KOCH-WESER and R. A. WILLIAMS, J. Am. med. Ass. *206*, 154 (1965).
[7] G. H. LATHE and M. WALKER, Biochem. J. *70*, 705 (1958).
[8] G. A. LEVVY and I. D. E. STOREY, Biochem. J. *44*, 295 (1949).
[9] I. H. STEVENSON and G. J. DUTTON, Biochem. J. *82*, 330 (1962).
[10] T. HARGREAVES, Clin. chim. Acta *11*, 278 (1965).