Laetrile: A Study of its Physicochemical and Biochemical Properties

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ABSTRACT

A study was made of the composition and biochemical behaviour of the drug, Laetrile, distributed for clinical trial in the United States and Canada. It was established that the Canadian and the American product are different pharmaceutical formulations, displaying different physicochemical and biochemical properties. The investigation demonstrated, furthermore, that neither preparation can be considered as a palliative in cancer therapy on the basis of the biological rationale advanced by their manufacturers.

SOMMAIRE

Les auteurs procèdent à l'étude de la composition et du destin biochimique du Laetrile, médicament distribué, pour essais cliniques, aux Etats-Unis et au Canada. Il a été établi que le produit canadien et l'américain ont des formules différentes et que leurs propriétés physicochimiques et biochimiques sont différentes. L'enquête a permis de démontrer, par ailleurs, qu'aucun des deux produits ne peut être considéré comme un palliatif du traitement anti-cancéreux, en se basant sur les considérations avancées par les fabricants.

LAETRILE, a drug manufactured and distributed until recently for clinical trial in Canada and the United States to determine its value as a palliative in cancer therapy, has been the subject of considerable controversy.[1-6] Its fundamental biological rationale, according to proponents of the preparation, derives from the Unitarian or Trophoblastic Thesis of Cancer (UTTC), announced in 1902 by John Beard of Edinburgh, Scotland.[7] According to this thesis, "the wandering germ cells of early life can later be activated to divide and produce trophoblast cells which, outside the canalization of pregnancy, are the malignant cells. Since the pregnancy trophoblast begins to disappear about the time the fetal pancreas develops, he [John Beard] also suggested the use of pancreatic extracts for the treatment of human malignancy."[1, 2] Later, H. H. Beard, expanding these concepts, postulated that cancer is a chymotrypsin and nutrition deficiency disease, and that "pancreatic chymotrypsin prevents about 80 per cent of civilization from ever developing malignancy, while in the other 20 per cent, benign or malignant tumours will always arise *unless prevented by adequate screening tests and chemotherapy*".[8]

As a chemotherapeutic cancerocidal agent, Laetrile—containing a glucoside obtained from bitter almond or peach kernels, generally known as amygdalin—was believed to release, in the presence of β-glucuronidase (an enzyme occurring in malignant neoplasms), sufficient amounts of hydrogen cyanide to stop tumour respiration and thus destroy cancerous tissue with some degree of specificity.

The object of this communication is to report a study concerning the composition and cytotoxic action of two formulations of this drug. For the purposes of this paper, one of these, manufactured in the United States, is referred to as Laetrile (U.S.) while the other, manufactured in Canada, is designated Laetrile (Can.). Both formulations were distributed under the same name, "Laetrile", and recommended for the same use, namely, palliative therapy in cancer.

PART I. PHYSICOCHEMICAL DATA

A. *Laetrile Manufactured in the United States*

Appearance, Optical Rotation and pH

The product was an amorphous solid. It failed to exhibit a sharp melting point, sintering gradually from 170 to 188° C. Dissolved in distilled water it displayed an optical rotation of −39° (C = 1% w/v), and a pH of 6.8 (C = 10% w/v).

Spectral Analysis

The ultraviolet absorption spectrum of the product is shown in Fig. 1(A). Maxima observed at 267, 261 and 256 mμ were generated by the major component present—amygdalin—and strong absorption beyond 250 mμ was found to be due to the occurrence of iodide ions in the formulation.

The infrared absorption spectrum of the product is reproduced in Fig. 2(A). It identified the major

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component of the formulation—amygdalin—and revealed admixture with additional constituents.

Chemical Analysis

Iodometric titration of the product with 0.02 N potassium iodate showed that it contained approximately 2.5% of iodide, and direct non-aqueous titration with 0.05 N perchloric acid established the presence of a basic component in equivalent concentration. In order to determine the identity of the basic moiety the drug was steam-distilled from alkaline solution into dilute hydrochloric acid. The infrared spectrum of the crude product obtained following removal of solvent under reduced pressure is shown in Fig. 2B. Purification of the isolate by fractional crystallization from acetone-ether yielded practically pure di-isopropylammonium chloride as illustrated in Fig. 2C. Similar treatment of amygdalin resulted in the formation of ammonium chloride (> 90% yield). The reactions observed may be considered to proceed as follows:

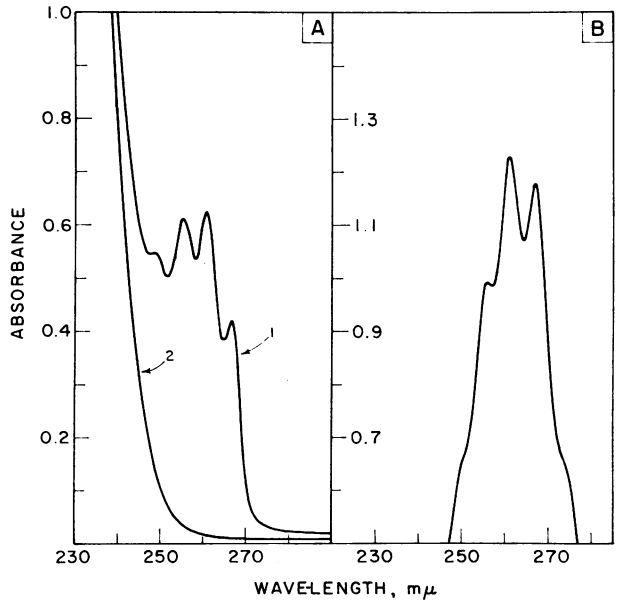

Fig. 1.—Ultraviolet absorption spectra: A1.—Laetrile (U.S.), 1.0 mg./ml. H_2O; A2.—Potassium iodide, 3.66 mg./100 ml. H_2O; B.—Laetrile (Can.), diluted 100X with H_2O (1.0 mg./ml.) on 0.5-1.5 absorbance scale.

(a) *Hydrolysis of Amygdalin*

(i) $C_6H_5—\underset{\underset{CN}{|}}{\overset{\overset{H}{|}}{C}}—O—C_6H_{10}O_4—O—C_6H_{11}O_5 + NaOH + H_2O$ →

Amygdalin

(D-Mandelonitrile-β-D-glucosido-6-β-D-glucose)

$NH_3\uparrow + C_6H_5—\underset{\underset{COONa}{|}}{\overset{\overset{H}{|}}{C}}—O—C_6H_{10}O_4—O—C_6H_{11}O_5$ ←

(ii) $NH_3 + HCl \rightarrow NH_4Cl$

(b) *Hydrolysis of Laetrile (U.S.)*

(i) $C_6H_5—\underset{\underset{CN}{|}}{\overset{\overset{H}{|}}{C}}—O—C_6H_{10}O_4—O—C_6H_{11}O_5 + [(CH_3)_2CH]_2 . NH . HI + 2NaOH$ ——→

Amygdalin — Di-isopropylammonium iodide

$C_6H_5—\underset{\underset{COONa}{|}}{\overset{\overset{H}{|}}{C}}—O—C_6H_{10}O_4—O—C_6H_{11}O_5 + [(CH_3)_2CH]_2 . NH\uparrow + NH_3\uparrow + NaI$ ←——

(ii) $[(CH_3)_2CH]_2 . NH + NH_3 + 2HCl \rightarrow [(CH_3)_2CH]_2 . NH . HCl + NH_4Cl$

Di-isopropylammonium chloride — Ammonium chloride

Thus the product of Laetrile hydrolysis represented a binary mixture composed of di-isopropylammonium chloride and ammonium chloride which was separated by fractional crystallization from acetone-ether.

Thin-layer chromatography confirmed the spectrophotometric data and established, moreover, the presence of 8 ± 2% of sucrose (adsorbent-silica gel G Merck; solvent—methylene dichloride:methanol: formamide 160:38:2; spray reagent-vanillin (5%) in concentrated sulfuric acid).

B. *Laetrile Manufactured in Canada*

Appearance, Optical Rotation and pH

The product was a colourless liquid with a pH of 3.9. Diluted with distilled water it displayed an optical rotation of −42.1° (C = 10% w/v).

Spectral Analysis

The ultraviolet absorption spectrum of the product is shown in Fig. 1B. Maxima observed at 267, 261 and 256 mμ were due to the presence of amygdalin in the formulation. Absorbance ratios were, however, not identical to those of a genuine reference standard assayed similarly. It was found that the discrepancies were attributable to the presence of phenol, a compound exhibiting intense absorption throughout the 255-275 mμ region.

The glucoside was isolated from the preparation following removal of the phenol by ether extraction,

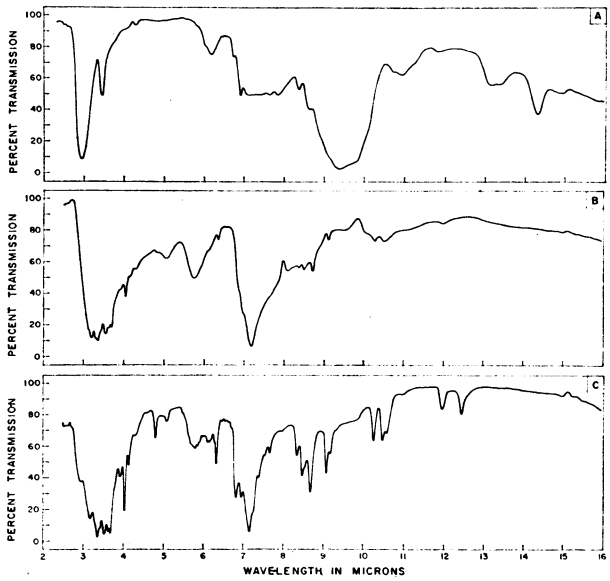

Fig. 2.—Infrared absorption spectra (0.3% in KBr): A.—Laetrile (U.S.); B.—Crude hydrolysis product; C.—Purified hydrolysis product.

evaporation of the aqueous phase and repeated crystallization of the residue from aqueous acetone.

Chemical Analysis

Examination of the product in accordance with the procedures described proved the absence of both iodides and of any volatile basic components.

Thin-layer chromatography and x-ray diffraction analyses (Fig. 3) confirmed these observations and established, furthermore, the absence of sucrose.

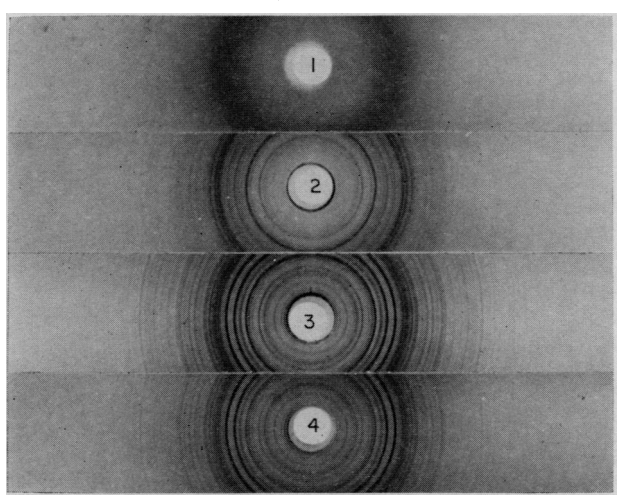

Fig. 3.—X-ray diffraction patterns: 1.—Laetrile (U.S.)—amorphous solid; 2.—Laetrile (U.S.)—recrystallized from aqueous acetone; 3.—Amygdalin—reference standard; 4.—Laetrile (Can.)—crystalline isolate.

The experimental data assembled are summarized in Table I. They illustrate clearly that the two preparations are different pharmaceutical formulations.

TABLE I.—COMPOSITION OF LAETRILE (U.S.) AND LAETRILE (CAN.)

Physical criteria and composition	*Laetrile*	
	Canada	*U.S.*
Label identification	A β-Cyanogenetic glucoside	A β-Cyanogenetic glucoside
Appearance	Amorphous solid	Colourless solution
Optical rotation	−39.0°	−42.1°
pH (10% in H_2O)	6.8	3.9
Amygdalin	87 ± 2%	98 ± 2%
Di-isopropylammonium iodide	5%	Absent
Phenol	Absent	0.5%
Sucrose	8 ± 2%	Absent
l-Mandelonitrile-β-glucuronoside (1)	Absent	Absent
Mandelonitrile-glucuronoside di-isopropylammonium salt*	Absent	Absent

*Literature distributed by manufacturer.

PART II. BIOCHEMICAL DATA

The compositional differences determined for Laetrile manufactured in the United States and Canada, respectively, were reflected in marked biochemical differences observed when evaluating both drugs as potential anticancer agents.

Experimental Method

The method, based principally upon incubation of surviving tumour tissue (slices or cell suspensions) in Warburg manometric vessels in the presence of radioactive tracers (amino acids, purines), has been previously described.[9, 10] During the incubation period (two hours), the tumour respiration (± glucose) and glycolysis were measured. After incubation the tissue material was separated into protein, ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) fractions, and the incorporation of tracers—indicating the rates of respective syntheses—was assayed. The effects of drugs on these metabolic parameters were then assessed in comparison with control values, obtained with the same tumour material in the absence of drugs.

Results

The results obtained with the two preparations [Laetrile (U.S.) and Laetrile (Can.)] and with sodium cyanide (NaCN) are summarized in Tables II and III, respectively.

Table II shows the observed effects on tumour energy metabolism, i.e. on respiration in the presence and absence of glucose, and on aerobic and anaerobic glycolysis. As can be seen, neither of the Laetriles at 4mM. concentration had any significant effect on these parameters, whether in primary human tumours or in Ehrlich ascites carcinoma cells. NaCN, however, at much lower concentration (0.1mM.) (Table II, Expt. 6 and 7), almost completely inhibited respiration of a human

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TABLE II.—EFFECTS OF LAETRILES AND NaCN ON ENERGY METABOLISM OF PRIMARY HUMAN TUMOURS AND ANIMAL ASCITES TUMOURS *in Vitro*

Exp. No.	*Neoplasm and drugs added*	$QO_2(-G)$	$QO_2(+G)$	$\frac{O_2}{QCO_2}$	$\frac{N_2}{QCO_2}$
1	Adenocarcinoma of breast (human)	0.6	0.6	1.3	—
	+Laetrile (U.S.) 4mM.	0.6	0.5	1.2	—
	+Laetrile (Can.) 4mM.	0.4	0.3	1.4	—
2	Astrocytoma (human)	2.6	2.6	1.0	3.9
	+Laetrile (U.S.) 4mM.	2.8	2.3	1.1	4.1
	+Laetrile (Can.) 4mM.	2.8	2.3	1.1	4.4
3	Metastases of bronchogenic carcinoma (human)	2.9	2.8	1.8	3.0
	+Laetrile (U.S.) 4mM.	2.5	2.6	1.8	3.4
4	Ehrlich ascites carcinoma.	9.8	7.2	16.7	44.0
	+Laetrile (Can.) 4mM.	9.8	6.5	20.0	45.3
	+benzaldehyde 0.4mM.	9.7	6.3	17.5	46.3
5	Ehrlich ascites carcinoma.	—	—	17.2	44.5
	+Laetrile (U.S.) 4mM.	—	—	18.5	42.2
	+Laetrile (Can.) 4mM	—	—	22.3	40.7
	+phenol 0.01%	—	—	22.3	42.6
6	Adenocarcinoma of colon (human)	2.2	2.6	5.5	8.6
	+NaCN 0.1mM.	0.2	—	7.3	—
	+5-fluorouracil 4mM.	—	—	4.8	—
	+methotrexate 0.1mM.	—	—	5.3	—
7	Novikoff ascites hepatoma	10.5	6.7	29.8	59.3
	+NaCN 0.1mM.	1.3	0.5	43.5	55.0

adenocarcinoma of the colon and of Novikov ascites hepatoma. NaCN also stimulated the aerobic glycolysis of both tumours to almost anaerobic levels.

Table III shows the drug effects on protein, RNA and DNA synthesis as measured by L-leucine-1-C^{14} and adenine-8-C^{14} incorporation into respective fractions. Again, except for inhibition of DNA synthesis by Laetrile (Can.), no significant inhibition could be observed either in human or animal tumours. On the other hand, NaCN, aerobically in the absence of glucose, inhibited almost completely all three syntheses, both in human and in animal tumour. Such inhibition, however, was almost absent, aerobically and anaerobically, when NaCN was added to vessels containing glucose. Apparently the NaCN effect on respiration was compensated by the increase in aerobic glycolysis as recorded in Table II.

The specific effects of Laetrile (Can.) on DNA syntheses could not be correlated with known effects of HCN. For an explanation the effects of benzaldehyde (possibly released from amygdalin on hydrolysis) and phenol (present in 0.5% concentration in Laetrile [Can.]), were investigated. As can be seen from experiments 4 and 5 in Tables II and III, benzaldehyde was without any effect, while phenol, in equivalent concentration, brought about exactly the same inhibition of DNA synthesis as did Laetrile (Can.) itself.

DISCUSSION AND CONCLUSIONS

The experimental data obtained indicate that Laetrile had no significant effect on cancer cells from primary human tumours or from animal ascites during two-hour incubation in physiological media. The 4mM. concentration used in the experiments may have caused in some tumours, in the absence of glucose, a small, non-specific (possibly osmotic) effect which is, however, in no way comparable to that obtained with only 0.1mM. NaCN. The somewhat stronger effects observed with Laetrile (Can.), especially on DNA syn-

TABLE III.—EFFECTS OF LAETRILES AND NaCN ON PROTEIN AND NUCLEIC ACID SYNTHESIS IN PRIMARY HUMAN TUMOURS AND ANIMAL ASCITES TUMOURS *in vitro*

L-leucine-1-C^{14} 2 mM.; 2 x 10^5 c./min./vessel; Adenine-8-C^{14} 0.1mM.; 6 x 10^5 c./min./vessel

			+counts/min./mg./dry tissue								
			In oxygen (-glucose)			*In oxygen (+glucose)*			*In nitrogen (+glucose)*		
Exp. No.	*Neoplasm and drugs added*		*Prot.*	*RNA*	*DNA*	*Prot.*	*RNA*	*DNA*	*Prot.*	*RNA*	*DNA*
1	Adenocarcinoma of breast		14	17	1.1	14	24	1.2	—	—	—
	+Laetrile (U.S.)	4mM.	11	16	1.1	16	20	1.1	—	—	—
	+Laetrile (Can.)	4mM.	8	16	0.7	13	22	0.9	—	—	—
2	Astrocytoma		4	17	1.4	5	22	0.8	2	7	0
	+Laetrile (U.S.)	4mM.	5	15	1.7	5	20	2.0	2	7	0
	+Laetrile (Can.)	4mM.	3	14	1.0	5	20	1.0	2	8	0
3	Metastases of bronchogenic carcinoma		23	34	2.9	85	68	7.4	15	11	1.4
	+Laetrile (U.S.)	4mM.	17	25	2.0	84	61	7.0	11	8	1.2
4	Ehrlich ascites carcinoma		208	412	39	352	1410	121	215	1368	118
	+Laetrile (Can.)	4mM.	171	369	27	388	1512	74	258	1270	23
	+benzaldehyde	0.4mM.	220	335	50	338	1410	134	260	1470	143
5	Ehrlich ascites carcinoma		—	—	—	277	1677	223	308	1742	201
	+Laetrile (U.S.)	4mM.	—	—	—	249	1795	190	298	1672	208
	+Laetrile (Can.)	4mM.	—	—	—	217	1748	114	233	1636	37
	+phenol	0.01%	—	—	—	230	1728	128	256	1670	46
6	Adenocarcinoma of colon		47	113	14	87	231	24	43	154	13
	+NaCN	0.1mM.	3	6	1	48	216	21	—	—	—
	+5-flurouracil	4mM.	—	—	—	71	216	17	—	—	—
	+methotrexate	0.1mM.	—	—	—	60	222	13	—	—	—
7	Novikoff ascites hepatoma		374	405	34	481	3900	412	482	3950	337
	+NaCN	0.1mM.	4	10	3	438	3512	361	450	3650	357

thesis, can be fully explained by the toxic effects of phenol added to this preparation. The results obtained with NaCN agree well with observations already reported[11, 12] which have shown that cyanide is not cancerocidal as long as glucose is available. Therefore, even if Laetrile were hydrolysed along the lines claimed by its manufacturers, the biological evidence indicates that it would be ineffective as a clinical anticancer agent, by virtue of cyanide release.

This study has demonstrated how compositional variations of a pharmaceutical formulation—considered to be minor ones by the manufacturer—brought about a dosage form displaying entirely different sets of biochemical characteristics. This phenomenon is known and recognized by some segments of the industry,[13] but its importance remains to be more fully appreciated by the industry at large. From the data obtained neither product can be considered as a palliative in cancer therapy on the basis of the biological rationale advanced by the manufacturers.

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