Toxic Substances in Cancer

VII. Effect on the Excised Frog Heart

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Following Maki's investigation,¹⁾ in which it was demonstrated that the aqueous extract of the tissue of gastric carcinoma contains a toxic substance inhibiting the excised frog heart, the authors studied the substances showing similar effect in the alcohol extract of the tissue, as well as in gastric juice of patients with gastric carcinoma.

EXPERIMENTAL WITH COMMENTS

Method

The frog used was *Rana nigromaculata*, weighing 14 to 23 g. An excised frog heart was irrigated first with Ringer's solution for frogs, then with a test solution (Ringer's solution containing a test material at given concentration) and the changes in state of beats were recorded kymographically. Criterion for determining the effect of the material on the heart is shown in Fig. 1.

Fig. 1. Schema of a kymogram with the explanation of the signs showing the grade of inhibitory effect. A_0 , amplitude before administration of the material ; A , minimal amplitude after the administration.

Materials and Results

Fractions of the tissue of gastric carcinoma soluble in organic solvents

Portions of the materials, which the authors had prepared in the previous work²⁾ to test the effect on the movement of *Paramecium*, were used here.

The results are summarized in Table I. Of all the materials tested, liquid acid fraction of the gastric carcinoma tissue inhibited the heart most remarkably, showing marked differences in intensity from the corresponding fraction of the normal gastric mucosa (Fig. 2).

TABLE I
Inhibitory Effects of the Alcohol Extract and its Fractions of the
Gastric Carcinoma Tissue on the Excised Frog Heart*

Material	Dilution (1:)							
	100	250	500	1000	2000	4000	8000	16000
Alcohol extract	≡ (≡)	≡ (≡)	+	+	±	±	-	-
Acetone soluble fraction	≡ (≡)	≡ (≡)	++	+	+	±	-	-
Acetone insoluble fraction	≡ (≡)	+	±	±	-	-	-	-
Liquid acid	≡ (≡)	≡ (≡)	++	+	+	±	±	-
Solid acid	≡ (≡)	≡ (≡)	++	+	+	±	-	-
Neutral fat	+	+	±	±	-	-	-	-
	(≡)	(+)	(+)	(+)	(-)	(-)	(-)	(-)

* Figures in parentheses show the results with the corresponding substances from the normal gastric mucosa.

Fig. 2. Kymograms showing the effect of the liquid acid fraction obtained from the tissue of gastric carcinoma (left) or of normal gastric mucosa (right).

Gastric juice of patients with gastric carcinoma

1) Dried material. Gastric juices obtained from patients with gastric carcinoma were filtered, condensed under reduced pressure, dried to powder and combined together. The control material was prepared, in the same manner, from the gastric juices of patients with surgical diseases other than malignant neoplasms.

As shown in Table II, these dried materials inhibited the heart only at high concentrations.

Then, the dried materials were fractionated in a manner as shown in Table III and each fraction was tested for the effect on the heart. The results are summarized in Table II. No remarkably effective substance, as

TABLE II
Inhibitory Effects of the Dried Powder and its Fractions of the
Gastric Juice of Patients with Gastric Carcinoma on
the Excised Frog Heart*

Material	Dilution (1:)				
	100	250	500	1000	2000
Dried powder	## (##)	## (##)	— (—)	— (—)	— (—)
Dialyzate	## (##)	+	+	—	—
Alcohol insoluble fraction	## (##)	+	+	—	—
Alcohol soluble fraction	## (##)	+	+	—	—
Acetone insoluble fraction	+	±	—	—	—
Acetone soluble fraction	## (##)	+	—	—	—
Dialyzed substance	± (—)	— (—)	— (—)	— (—)	— (—)
K ₁	## (—)	+	—	—	—
K ₂	+	±	—	—	—
K ₃	+	±	—	—	—
K ₄	+	±	—	—	—
K ₅	— (—)	— (—)	— (—)	— (—)	— (—)
K ₆	+	+	—	—	—
	(—)	(—)	(—)	(—)	(—)

* Figures in parentheses show the results with the corresponding substances obtained from the gastric juice of patients with surgical diseases other than malignant neoplasms.

TABLE III
Scheme for Fractionating the Dried Powder of the Gastric Juice

much effective as the ether extract of fresh gastric juice which will be described below, was obtained by this method, though it was found that the fractions prepared from the dialyzate of the dried material were generally more intense in inhibitory effect than those derived from the dialyzed substance.

2) Ether extract. Gastric juices were fractionated systematically in the manner in which the authors had previously followed after the *Paramecium*-immobilizing substance in the gastric juice of patients with gastric carcinoma.³⁾

An outline of the method is as follows: Fresh gastric juice was agitated in a fractionation funnel with an equal volume of ether and the ether extract was obtained by drying the upper ethereal layer by the aid of a fan. Pooled ether extract was then treated with acetone to be fractionated into soluble and insoluble fractions. A portion of the acetone-soluble fraction was neutralized with sodium methoxide and separated from neutral fat. Fatty acids thus isolated were then converted to lead salts and divided into two fractions: liquid acid, whose Pb salt is insoluble in alcohol, and solid

acid, whose salt is soluble in this solvent.

In Table IV are shown the effects of these fractions on the excised frog heart. Ether extract of the gastric juice of patients with gastric carcinoma was much more effective than the control extract, the highest possible dilution of the former to inhibit the heart being eight times as much as that of the latter.

TABLE IV
Inhibitory Effects of the Ether Extract and its Fractions of the
Gastric Juice of Patients with Gastric Carcinoma
on the Excised Frog Heart*

Material	Dilution (1:)								
	100	250	500	1000	2000	4000	8000	16000	32000
Ether extract	## (##)	## (##)	## (##)	++ (+)	± (±)	± (-)	± (-)	± (-)	- (-)
Acetone soluble fraction	## (##)	## (##)	## (+)	± (-)	± (-)	± (-)	± (-)	- (-)	- (-)
Acetone insoluble fraction	± (+)	± (+)	± (-)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)
Liquid acid	## (##)	## (##)	++ (+)	± (+)	± (+)	± (±)	± (-)	± (-)	- (-)
Solid acid	## (##)	## (##)	± (+)	± (+)	± (+)	- (-)	- (-)	- (-)	- (-)
Neutral fat	## (##)	± (+)	± (+)	± (+)	± (±)	- (-)	- (-)	- (-)	- (-)

* Figures in parentheses, see Table II.

Fig. 3. Kymograms showing the effect of the liquid acid fraction separated from the gastric juice of patients with gastric carcinoma (left) or of patients with surgical diseases other than malignant neoplasms (right).

Effective substance in the ether extract of cancer material was soluble

in acetone. Of the three materials prepared from the acetone-soluble fraction of the extract, liquid acid fraction presented the most intense inhibition, more marked at various concentrations than that of the corresponding control fraction (Fig. 3).

SUMMARY

1. Liquid acid fraction isolated from the alcohol extract of the tissue of gastric carcinoma inhibited the excised frog heart more markedly than the corresponding fraction of the normal gastric mucosa.

2. Dried powder of gastric juice of patients with gastric carcinoma and many of its fractions showed only slight inhibition to the heart.

3. Ether extract of fresh gastric juice of patients with gastric carcinoma inhibited the heart intensely while the control extract was far less effective. Of all the fractions of the extract, liquid acid fraction was the most effective in inhibition.

References

- 1) Maki, Tohoku J. Exper. Med., 1956, **63**, 121.
 - 2) Yamaguchi, Kamma & Oh-Uti, *ibid.*, this volume, p. 185.
 - 3) Oh-Uti, Abe & Yamaguchi, *ibid.*, 1957, **67**, 91.
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