AN UNSATURATED FATTY ACID FRACTION OF PIG PANCREAS WHICH INHIBITS THE GROWTH OF CHICKEN SARCOMA

By O. M. HELMER, Ph.D.

(From the Lilly Laboratories for Clinical Research, Indianapolis City Hospital, and the Lilly Research Laboratories, Indianapolis)

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From time to time articles have appeared in the literature showing that the higher fatty acids exert an inhibiting action on tumor growth. Webb (1) in 1901 used soap solution in the treatment of human tumors. On the basis of Webb's work Shaw-Mackenzie (2) and Gardner (3) reported on the use of sodium oleate in the treatment of cancer. Nakahara (4) found that unsaturated fatty acids when injected intraperitoneally caused an increased resistance to the growth of subsequently implanted Bashford adenocarcinoma. Similarly, the growth of autografts of spontaneous tumors was retarded. Bierich (5) also reported an increased resistance to cancer implantation by means of an unsaturated fatty acid. Leclux (6), in a paper in which he gave a comprehensive review of lipids in relation to cancer, reported that sodium oleate, oleic acid, and stearic acid when applied locally in intervals between painting retarded the appearance of tar cancer and that an iron salt of oleic acid exerted the same effect when injected into the peritoneum.

The growth of chicken tumors also is affected by the unsaturated fatty acids. Begg and Aitken (7) reported that intratumoral injections of sodium oleate sometimes led to regression and occasionally to complete disappearance of tumors and also that potent Rous filtrates were found to be inactivated by the addition of a neutralized solution of sodium oleate. Firie (8), in studying the inhibiting action of pancreatic extracts on the Rous and Fujinami tumors, concluded that the inactivating factor of the pancreatic extracts was associated with the fatty acid and lecithin fractions. Baker and McIntosh (9) and Sugiura (10) also found that aqueous extracts of pancreas inhibited the chicken sarcoma, and Vassiliadis (11) mentioned that organo-extracts of pancreas had a retarding action on tar tumors.

It is the purpose of this paper to present a stepwise fractionation of fresh pancreatic tissue which demonstrates that the inhibiting action of this tissue against the Rous chicken sarcoma is found principally in the unsaturated fatty acid fraction.
Materials and Methods

The chicken sarcoma used in these experiments was Chicken Tumor I, Rockefeller Institute series.\(^1\) Frozen pig pancreas was used in all of the experiments because pig pancreas was found to be the richest source of the inhibiting agent. Moreover, it was also found to be unnecessary to dry the pancreas before extraction.

The chicken tumor extracts were prepared from desiccated tissue. 1 gm. of the powdered desiccate was extracted with 60 cc. of sterile distilled water. During the extraction the reaction was adjusted to pH 7.2–7.4 by the addition of 0.1 N NaOH. The debris was removed by centrifugation and the supernatant fluid filtered through coarse paper.

The extracted pancreatic lipids to be tested were dissolved in ether and a known amount was pipetted into a 15 cc. centrifuge tube. The ether was removed by immersing the tube in warm water and removing the last traces of ether by application of a vacuum. Then an appropriate amount of 1 per cent Na\(_2\)HPO\(_4\) was added, the mouth of the tube closed with cotton, and the tube boiled in a water bath for 15 minutes to disperse the lipids and for sterilization. While the tube was still hot the cotton was removed and a sterile rubber cap substituted. The tube was then shaken so that the fatty substance would form a fairly stable emulsion. The tube was cooled to room temperature, an equal volume of the chicken tumor extract was added, and the mixture was allowed to stand at room temperature until injected.

A control tube was similarly prepared, using an equal amount of a solution of 1 per cent Na\(_2\)HPO\(_4\).

The experimental mixtures and their controls were injected intradermally into the breasts of young Plymouth Rock hens. In each case the amount injected was 0.4 cc. Six to eight injections were made into the breast of each hen. To eliminate differences in susceptibility of the chickens the measurements of the experimental tumors were recorded when the control tumors were of a fixed size. The inhibiting activity of the various fractions is expressed as the amount of inhibitor necessary to inactivate completely 0.2 cc. of the 1:60 chicken tumor extract.

To obtain the data reported in this paper 825 injections were made into 127 chickens. For simplicity the results are presented in tabular form.

Fractionation.—Since frozen tissue was used as a starting material acetone and alcohol were tried as the preliminary extractives. Both proved satisfactory, but as acetone was preferable it was finally chosen for the first extraction. After removal of the acetone and water various solvents, such as benzene, ether, and acetone, were used in the second step of the fractionation. Acetone was again found to be the most suitable. The fractionation finally adopted is shown in Table I.

The unsaturated fatty acid fraction (9) had an iodine number of 89 and was an oil at room temperature. This fraction was also deeply pigmented, the pigment following the activity throughout the fractionation. It could not be further puri-

\(^1\) This was kindly supplied by Dr. James B. Murphy.
TABLE I

Fractionation of Pancreatic Tissue for the Isolation of a Tumor-Inhibiting Agent

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Inactive/Active</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Residue</td>
<td>Inactive</td>
<td>Evaporated to dryness (Weight 80 gm.)</td>
</tr>
<tr>
<td>2. Cold acetone insoluble residue</td>
<td>Inactive</td>
<td>Dissolved in 70% alcohol; cooled</td>
</tr>
<tr>
<td>4. 70% alcohol insoluble residue</td>
<td>Inactive</td>
<td>Dissolved in 70% alcohol; cooled</td>
</tr>
<tr>
<td>6. Nonsaponifiable alkaline ether extract</td>
<td>Relatively Inactive</td>
<td>Saponified with alcoholic KOH; the alcohol evaporated and 2 volumes of water added; extracted with ether in alkaline condition; then made acid to Congo red and again extracted with ether</td>
</tr>
<tr>
<td>7. Acid ether extract</td>
<td>Active</td>
<td>Evaporated to dryness (Weight 37.6 gm.)</td>
</tr>
<tr>
<td>8. Ether insoluble Pb soap</td>
<td>Relatively Inactive</td>
<td>Dissolved in alcohol; neutralized with KOH; precipitated with Pb acetate; precipitate dried in vacuo; extracted with ether</td>
</tr>
<tr>
<td>9. Ether soluble Pb soap</td>
<td>Active</td>
<td>Evaporated to dryness (Weight 21 gm.)</td>
</tr>
</tbody>
</table>

500 gm. frozen pig pancreas

Extracted 3 times with acetone
GROWTH-INHIBITING FRACTION OF PANCREAS

fixed by precipitation from organic solvents. Therefore the following procedures, which are not shown in the table, were utilized.

First, the unsaturated fatty acid fraction (9) was distilled under the full vacuum of the cenco hyvac pump. A light yellow oil weighing 14 gm. distilled over at a pressure of about 1 mm. and a temperature of 176°C. This oil in quantities of 0.25 mg. inhibited 100 per cent. The tumor activity was inactivated also by 0.25 mg. of the dark brown residue. The distillate was liquid at room temperature and solidified in the ice box; it had an iodine number of 90, which corresponds with that of a fatty acid having one unsaturated group. When dissolved in alcohol and titrated with alcoholic KOH 0.3705 gm. and 0.4514 gm. required for neutralization 12.99 cc. and 15.57 cc., respectively, of 0.1 N KOH. These figures correspond to a molecular weight of 286.7 and 282.8 as compared to 282.36 for the molecular weight of oleic acid. Therefore it appears that the iodine number, the molecular weight, and the physical properties compare quite closely with those of oleic acid.

Commercial oleic acid, when tested in the same way as the pancreatic fractions, was found to have almost the same inhibiting power as the pancreatic fractions; 0.25 mg. of oleic acid inhibited 100 per cent, whereas 0.10 mg. of oleic acid did not cause complete inhibition.

In a second procedure the unsaturated fatty acid fraction was dissolved in ether and extracted with 5 per cent NaOH in a separatory funnel. The alkaline aqueous phase was then removed, acidified with HCl, and re-extracted with ether. This procedure was repeated twice and the final ether extract washed with water, dried with sodium sulfate, and the ether distilled off. The material retained all of the pigment and inhibited completely in quantities of 0.25 mg.

Thirdly, a sample of the unsaturated fatty acid fraction was dissolved in ether and shaken with norit to remove the pigment. Most of the pigment was removed. The light yellow oil obtained by evaporation of the ether caused complete inhibition in quantities of 0.25 mg. Therefore one may say that although the pigment follows the activity throughout most of the fractionation it is in itself not the inhibiting agent. However, the last traces of pigment were not removed. Even on distillation the oil that came over had a light yellow color.

Since the active fraction had an iodine number of 90, a sample of the oil was hydrogenated using a platinum black catalyst. Hydrogenation completely destroyed the inhibiting action of the unsaturated fatty acid fraction. No inhibition was obtained with 5.0 mg., equivalent to 119 mg. of pancreas.

DISCUSSION

The results of these experiments show that the chicken sarcoma-inhibiting factor in pig pancreas is definitely associated with the un-

The author wishes to thank Dr. E. C. Kleiderer of the Lilly Research Laboratories for carrying out the hydrogenation.
saturated fatty acid fraction. The acid number, the iodine number, and the physical properties are similar to those of oleic acid. This does not necessarily mean that the inhibiting agent is oleic acid. Further purification will be necessary to clear up this point. Commercial oleic acid was found also to exert an inhibiting action against the chicken sarcoma agent in quantities comparable to the fractions isolated from the pancreas. Hydrogenation of the unsaturated fatty acid fraction destroyed its inhibiting properties. These results are in agreement with those of Pirie, who reported that oleic acid inactivated the Fujinami tumor filtrate but that stearic acid did not. However, our findings differ from those of Pirie in that the unsaturated fatty acid fraction was found to exert a markedly stronger inhibiting effect than did the phospholipoid fraction.

The high concentration of lipase in pancreatic tissue may explain the strong inhibiting action of pig pancreas. Without doubt the lipase is responsible for the large quantities of free fatty acid which may be liberated by autolysis after the death of the animal. However, it is interesting to note that sarcoma of the pancreas is rarely found at human postmortem examinations (12).

Along with the work of Begg and Aitken and of Pirie, the data presented in this paper definitely show that the unsaturated fatty acids are able to act directly on the tumor-inducing agent of chicken sarcoma as well as to increase the resistance to transplantable mouse tumors, as shown by Nakahara. More work will have to be done before any conclusions can be drawn as to the mechanism of the inhibiting action of the unsaturated fatty acid fractions.

SUMMARY

The inhibiting action of pancreatic tissue was found to be associated with the unsaturated fatty acid fraction. As small an amount of fatty acid as 0.1 mg. inhibited the chicken sarcoma agent contained in 0.2 cc. of a 1:60 aqueous extract of Chicken Tumor I. The unsaturated fatty acid had an acid number and an iodine number similar to those for oleic acid. Commercial oleic acid also was found to inhibit the growth of the chicken sarcoma in comparable quantities.

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BIBLIOGRAPHY