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## Failure of Wheat Germ Oil to Produce Neoplasms.

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Rowntree[1] and his associates claimed that intraperitoneal sarcomas developed in rats fed on a crude ether extracted wheat germ oil. The experiment has since been repeated in a number of laboratories with negative results. Those who have reported failure include Carruthers,[2] Day and his associates,[3] Evans and Emerson,[4] and Dingemanse and van Eck.[5] Because Rowntree continues[6] to maintain that sarcomas can be produced by this method, we wish to report our own experience in the matter.

We attempted to repeat as exactly as possible, the original experiment described by Rowntree. The wheat germ oil was prepared according to the method used by E. R. Squibb and Sons in making the oil used by Rowntree and Dorrance for their experiment. Our wheat germ was purchased from Pillsbury Flour Mills Co., in New York. Its extraction was carried out in 2 large Soxhlet extractors. The ether was boiled in a 2-liter round flask and condensed by a large water-cooled bulb condenser into the midsection of the extraction chamber which contained a large paper thimble holding approximately one-third of a pound of wheat germ. The extraction chamber was allowed to siphon over 2 or 3 times, before inserting a fresh supply of wheat germ in the thimble. In this manner 750 pounds of wheat germ were extracted with 267 liters of ether. The ether used was that put up in copper lined cans for use as an anesthetic by E. R. Squibb and Sons. This was the same form of ether

1 Rowntree, L. G., Steinberg, A., Dorrance, G. M., and Ciccone, E. F., *Am. J. Cancer*, 1937, **31**, 359.

2 Carruthers, C., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 107.

3 Day, H. G., Becker, J. E., and McCollum, E. V., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 21.

4 Evans, H. M., and Emerson, G. A., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 318.

5 Dingemanse, E., and van Eck, W. F., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 622.

6 Rowntree, L. G., Steinberg, A., and Brown, W. R., Paper read at the 32nd Annual Meeting of the American Association for Cancer Research, Richmond, Virginia, April 6, 1939.

used in the extraction of the wheat germ oil prepared for Rowntree by E. R. Squibb and Sons.* This form of ether is generally considered to be peroxide-free.

The extract thus obtained was filtered through filter paper to remove the fine particles of wheat germ, and washed with one-half volume of 4% sodium hydroxide solution. It was then washed 4 or 5 times with water. A clear solution resulted. This was distilled at 36° at atmospheric pressure. When most of the ether had been removed the solution was distilled in a vacuum, and a stream of carbon dioxide passed through the oil to remove the last traces of the ether. The temperature was finally increased to 60° to 70° for 1 to 2 hours.

The oil obtained was perfectly clear. It was stored in a refrigerator. After 24 hours of standing in the cold an insoluble precipitate formed. Before the oil was used this material that had separated out was stirred up and dissolved at room temperature, and the whole fed to the rats.

A total of 15 liters of wheat germ oil were extracted in this manner from approximately 337,000 g of wheat germ, during the period from February to May, 1938. The yield, it will be noted, was approximately 4%. The task was a tedious one, but was carried out with all possible care.

Twenty male rats of the Wistar strain, which was the same strain that Rowntree used, were fed the oil in the manner which gave him the best success. Four cubic centimeters of it were poured daily over the portion of food consumed by each rat. The food was a stock diet containing the following ingredients: rolled oats 15 parts; cracked corn 60 parts; dried meat scraps 14 parts; powdered milk 10 parts; sodium chloride 1 part. To this was added 1.25% of cod-liver oil by weight. Water was given freely in drop bottles.

The feeding of the wheat germ oil was begun on July 27, 1938, and was ended when the supply of oil was exhausted on March 28, 1939. Four of the rats died during the course of the experiment, one on February 2, 1939, another on February 28, 1939, and two on March 28, 1939. Autopsy showed pneumonia to be the cause of death in each instance. There were no sarcomas in the peritoneal cavity or elsewhere.

The remaining 16 rats were sacrificed on July 22, 1939. No sarcoma was found in any of them. The only remarkable feature at autopsy was the unusual amount of fat in the omentum, mesentery, and retroperitoneal regions.

* Private communication from Dr. Anderson of E. R. Squibb and Sons.

*Summary.* A careful repetition of Rowntree's experiment in which ether extracted wheat germ oil was fed to rats failed to confirm his claim that intraperitoneal sarcomas can be induced in this manner.

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## Action of a Quaternary Ammonium Type of Wetting Agent on Metabolism of Microörganisms Associated with Dental Caries.

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In the search for a compound which can effectively inhibit the metabolism of microörganisms found in lesions of dental caries, or in plaques associated with such lesions, we have studied the action of alkyl dimethyl benzyl ammonium chloride (Zephiran*) on these bacteria. The germicidal action of this compound was described by G. Domagh.[1]

Pure cultures of 5 microörganisms, which are found constantly or very frequently in association with human dental caries, were studied. These were a lactobacillus, 2 strains of *M. tetragenus, Staphylococcus albus,* an unidentified, aerobic, acid-producing, Gram-positive diplococcus and a yeast of the genus Monilia. Washed suspensions of the organisms were placed in appropriate buffers containing 0.02M glucose, and their rate of respiration or glycolysis measured in Warburg vessels. The alkyl compound exerts a pronounced inhibitory effect on both respiration and glycolysis at extremely low dilutions (concentrations estimated at M/10,000 to M/50,000).† At a concentration of M/10,000 the compound exerts its full effect on one billion cells within 5 to 10 minutes. That the inhibition is complete and irreversible is demonstrated by the following experiment: organisms which were exposed to the compound for several minutes, and then centrifuged off and washed with

* The authors are indebted to the Alba Pharmaceutical Company for the Zephiran compound employed in this study.

1 Domagh, G., *Deutsche Med. Wochenschr.*, 1935, **61**, 829.

† The molecular weight of the alkyl dimethyl benzyl ammonium chloride compound was approximated as 350 by taking an arithmetic average of the alkyl groups which vary from $C_8$ to $C_{18}$. The "compound" is a mixture of the quaternary ammonium derivatives of fatty acids obtained from coconut oil.