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A Cancerogenic Extract from Human Bile and Gall Bladders.*

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The possibility that human bile might contain substances which have cancerogenic activity was suggested by the discovery that a powerful cancerogen, methylcholanthrene, could be made from bile acids,[1-3] by the incidence of carcinoma of the biliary system, where comparatively few cells give rise to numerous tumors, and by discovery that extracts of human liver have cancerogenic activity.[4] Some of this material might be excreted in the bile and induce tumors in the biliary passages, and in the gastrointestinal tract.

Human bile has not previously been reported to induce sarcomas at the site of injection. Using ox bile, Turner[5] induced 1 sarcoma in 75 mice that lived over 34 weeks. Bürger and Uiker[6] induced only leukemia-like changes in the liver and spleen of mice with an emulsion of human bile. Neufach and Shabad[7] also induced no tumors at the site of injection of a benzene extract of human bile, but stated that the incidence of various tumors in distant organs was greater than in controls.

Methods. Human gall bladder bile and some of the gall bladders from which it was obtained were collected from adults. Approximately 40% of these persons died with malignant tumors. The dried residue, weighing 2400 g, was saponified with alcoholic potassium hydroxide by refluxing on a steam bath for 24 hr. After repeated extractions with ethylene dichloride the extracts were combined and dried over anhydrous sodium sulfate. The extract was then filtered and the ethylene dichloride distilled in partial vacuum. The residue was resaponified with alcoholic potassium hydroxide for 4 hr, and then extracted, dehydrated, filtered, and distilled as before. The nonsaponifiable residue weighed 38.5 g.

This extract was tested for cancerogenic activity by subcutaneous injection. Fifty-three mice were each injected with 250 mg of the extract in 0.75 cc of sesame oil. The injections were repeated at 6 weeks. This sesame oil has not induced tumors in this stock of mice when injected alone or with various tissue extracts prepared by this method.[4,8,9] The mice were from 107 to 127 days old. Something about their susceptibility to spontaneous and induced tumors has been given in several reports.[4,9,10] They do not have spontaneous sarcomas, other than lymphosarcomas.

Results. The mice lived to the periods of

TABLE I.
Cancerogenicity of a Bile Extract.

Time in mo.	Living mice ♂	Living mice ♀	Living mice Total	Deaths with sarcoma
0	13	40	53	
6	6	39	45	
12	1	34	35	
15	0	30	30	1
18	0	25	25	
21	0	13	13	1
24	0	7	7	3
27	0	3	3	
29	0	2	2	

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1 Cook, J. W., and Haslewood, G. A. D., *J. Chem. Soc.*, 1934, p. 428.

2 Wieland, H., and Dane, E., *Ztsch. f. physiol. Chem.*, 1933, **219**, 240.

3 Fieser, L. F., and Newman, M. S., *J. Am. Chem. Soc.*, 1935, **57**, 961.

4 See review: Steiner, Paul E., *Cancer Research*, 1942, **2**, 425.

5 Turner, F. C., *U. S. Public Health Reports*, 1939, **54**, 1603.

6 Bürger, M., and Uiker, R., *Klin. Wchnschr.*, 1937, **16**, 334.

7 Neufach, S. A., and Shabad, L. M., *Bulletin de Biologie et de Médecine Experimentale de L'URSS*, 1938, **6**, 259.

8 Steiner, Paul, E., *Cancer Research*, 1942, **2**, 181.

9 Steiner, Paul E., *Cancer Research*, in press.

10 Steele, R., Koch, F. C., and Steiner, P. E., *Cancer Research*, 1941, **1**, 614.

time shown in the table. Five sarcomas occurred at the site of injection of the extract in mice dying in the 14th, 20th, 23rd, 23rd, and 24th months after the first injection. Two mice are alive and without visible tumors at 29 months. The tumors were spindle or spindle and mixed cell sarcomas. They resemble sarcomas induced by cancerogenic chemicals in the subcutaneous tissues in other experiments, both in their morphology and growth.

Comment. The occurrence of 5 sarcomas in 32 mice (percentage yield of 15.6) indicates, despite the long induction time, that this extract had a fair degree of cancerogenic potency. Each mouse was injected with the non-saponifiable lipid residue from a total of 31.05 g of dried gall bladder bile and dried gall bladders themselves. This represents a large volume of gall bladder bile and an even larger amount of unconcentrated bile. Other extracts of bile, prepared by different methods, either have not induced tumors or are still under test. They will be described in a later report.

Although Cook, Kennaway, and Kennaway[11] reported the induction of tumors with desoxycholic acid, Shear *et al.*[12] and others have failed to induce tumors with bile acids. It is highly improbable that the tumors reported here were induced by bile acids because the extract was made with a lipid solvent from a strongly alkaline aqueous-alcohol solution in which the bile acids should remain as salts.

11 Cook, J. W., Kennaway, E. L., and Kennaway, N. M., *Nature*, 1940, **145**, 627.

12 Shear, M. J., Leiter, J., and Perrault, A., *J. Nat. Canc. Inst.*, 1941, **2**, 99.

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"Folic Acid" in Nutritional Achromotrichia.

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The concept of synthesis of vitamins by the bacterial flora of the intestine seems to have originated in work on the phenomenon of refection.[1] Nutritional investigation of the problem received a great impetus with the announcement[2] that sulfaguanidine, an antibacterial agent which is poorly absorbed, reduced the intestinal flora.

Black *et al.*[3] demonstrated reduced growth rate in rats on purified diets containing synthetic vitamin mixtures and sulfaguanidine. Various other investigators confirmed and extended these observations.[4-9] The nature of the deficiencies produced by the inclusion of sulfonamides in synthetic diets has recently been disclosed. Black *et al.*[10] demonstrated effects by both vitamin K and p-aminobenzoic acid, the vitamin K correcting a hypoprothrombinemia and the para-aminobenzoic acid improving growth. Biotin deficiency in rats fed sulfonamide-containing

1 Guerrant, N. B., Dutcher, R. A., and Tomey, L. F., *J. Biol. Chem.*, 1935, **110**, 233.

2 Marshall, E. K., Jr., Bratton, A. Calvin, White, H. J., and Litchfield, J. T., Jr., *Bull. Johns Hopkins Hosp.*, 1940, **67**, 163.

3 Black, S., McKibbin, J. M., and Elvehjem, C. A., PROC. SOC. EXP. BIOL. AND MED., 1941, **47**, 308.

4 Mackenzie, J. B., Mackenzie, C. G., and McCollum, E. V., *Science*, 1942, **94**, 518.

5 Dann, W. J., *J. Biol. Chem.*, 1941, **141**, 803.

6 Daft, F. S., Ashburn, L. L., Spicer, S. S., and Sebrell, W. H., *U. S. Public Health Rep.*, 1942, **57**, 217.

7 Welch, A. D., *Fed. Proc.*, 1942, **1**, 171.

8 Light, R. F., Cracas, L. J., Olcott, C. T., and Frey, C. N., *J. Nutr.*, 1942, Nov., 24.

9 Martin, G. J., PROC. SOC. EXP. BIOL. AND MED., 1942, **51**, 56.

10 Black, S., Overman, R. S., Elvehjem, C. A., and Link, K. P., *J. Biol. Chem.*, 1942, **145**, 137.