designed to study the reverse process, or efflux from the intestine. When sacs were incubated for 10 min in micellar media containing labeled bile salt and cholesterol, and were subsequently transferred to unlabeled micellar media of the same composition, little exchange of isotope occurred; these data indicate that efflux or exchange of isotopes were not responsible for the observed ratios in the intestinal sac.

Summary. Data are presented on the absorption of cholesterol and other lipids by everted rat intestinal sacs from micellar solutions of sodium taurochenodeoxycholate. It appears that neither simple, two-component nor complex, multiple-component micelles penetrate the intestinal mucosal wall intact, but rather that the lipids and the bile salts are absorbed individually by rat intestine.

1. Dasher, G. F., Science **116**, 660 (1952).
2. Hofmann, A. F., Biochem. J. **89**, 57 (1963).
3. Hofmann, A. F., Biochim. Biophys. Acta **70**, 306 (1963).
4. Dowse, C. M., Saunders, J. A., and Schofield, B., J. Physiol., (London) **134**, 515 (1956).
5. Borgstrom, B., J. Clin. Invest. **39**, 809 (1960).
6. Hofmann, A. F. and Borgstrom, B., Federation Proc. **21**, 43 (1962).
7. Borgstrom, B., Gastroenterology **43**, 216 (1962).
8. Simmonds, W. J., Hofmann, A. F., and Theodor, E., J. Clin. Invest. **46**, 874 (1967).
9. Johnston, J. M. and Borgstrom, B., Acta Chem. Scand. **17**, 905 (1963).
10. Johnston, J. M. and Borgstrom, B., Biochim. Biophys. Acta **84**, 412 (1964).
11. Norman, A., Arkiv. Kemi, **8**, 331 (1955).
12. Horecker, B. L., Ma, T. S., and Haas, E., J. Biol. Chem. **136**, 775 (1940).
13. Wilson, T. H. and Wiseman, G., J. Physiol. (London) **123**, 116 (1954).

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Toxohormone (32760)

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Toxohormone was the name given by Nakahara and Fukuoka (1) to a toxic material extracted from tumor tissue. This toxic extract from tumor tissue would, upon injection into normal animals, lower liver catalase activity, decrease plasma iron concentration, and produce several other changes which are characteristic of the tumor-bearing animal (2, 3). Nakahara and Fukuoka were able to prepare this toxic extract from all growing cancer tissues tested but not from normal tissues (1). A number of investigations have indicated that active extracts can be prepared from several different normal tissues (4–7) and that all tumor tissues may not have greater activity than some normal tissues (7, 8). We observed that bacterial products gave activities similar to toxohormone and that extracts prepared from normal or tumor tissues were equally active in the absence of bacteria (7, 9). The toxic activity of bacteria carried through the toxohormone extraction procedures has been confirmed (10); but in several studies, it has been reported that toxohormone activity could be prepared from tumor tissues in the absence of bacteria (8, 10–12). These studies, however, did not include similar preparations made from normal tissues. Olivares *et al.* (13) showed that autolysis of normal tissues will give an extract with "toxohormone-like" activity. The extraction procedures employed for the preparation of toxohormone make it difficult to standardize and avoid the possibility of autolysis. In the experiments reported in this investigation, normal tissue was processed simultaneously with each tumor tissue preparation.

Materials and Methods. The tissues used were: Walker carcinoma 256 carried intramuscularly in Holtzman rats, 20-methylcholanthrene tumors induced intramuscularly in Fisher rats, normal liver, and normal tissue preparations which consisted of the whole rat minus skin, bones, and intestines. A portion of each tissue was inoculated into nutri-

ent broth and was discarded if any bacteria were found after 48 hours incubation at 37°C. Tissues described as fresh were used after being frozen for 48 hours. Another part of each tissue was dried at 70°C in a vacuum oven, duplicating the procedure used by Nakahara *et al.* (11) in their studies on germfree tumors.

The tissues were fractionated by the standard method for preparing crude toxohormone recommended by Hozumi *et al.* (14). In each preparation the normal tissue was processed simultaneously with the tumor tissue. The final dried extracts were suspended in water, 100 mg/ml, and injected intraperitoneally into normal rats weighing approximately 200 gm. Plasma iron concentration, shown to be a sensitive measure of these toxic extracts (15,16), was measured 16 hours later by the method of Schade *et al.* (17). Control animals were given 1 ml of water. All injections were given at 4 p. m.

The results reported represent 3 separate preparations of Walker tumor and 2 of 20-methylcholanthrene, each of which also included a normal tissue preparation. A portion of these tumor preparations were given further treatment as follows: the toxohormone was suspended in pyrogen-free water, 100 mg/ml, evaporated in an oven at 105°C until dry and then resuspended in water. This procedure did not noticeably alter solubility. All extracts remained in suspension when centrifuged at 2600*g* for 10 min. The carbon particles used for injection were heated for 3 hours at 170°C to make certain they were free of endotoxin.

Results. The lowering of plasma iron concentration which resulted from the injection of these extracts is shown in Table I. Preparations from both fresh tumor tissues lowered plasma iron concentration. Neither tumor extract, however, lowered the plasma iron more than did the normal tissues. Vacuum drying at 70°C of both the tumor and normal tissues gave a final extract with greater activity than the fresh tissue.

TABLE I. Plama Iron Concentration in Rats 16 Hours after an Intraperitoneal Injection of Preparations from Tumor or Normal Tissues.

Extract prepared from[a]	No. rats	Plasma iron (μg/100 ml)
Fresh Walker tumor[b]	21	183 ± 11[c]
Dried Walker tumor[d]	18	119 ± 5
Fresh 20-methylcholanthrene tumor	10	219 ± 9
Dried 20-methylcholanthrene tumor	8	154 ± 13
Fresh liver	7	139 ± 17
Fresh normal tissue[e]	14	130 ± 6
Dried normal tissue	18	108 ± 7
Injections of water only (1 ml)	23	273 ± 10

[a] 100 mg injected/rat; av wt. of rats was 200 gm.

[b] Fresh tissues have been frozen for 48 hours, during which time a check was made for bacterial contamination.

[c] Mean ± SE.

[d] Dried tissues were heated in a vacuum oven at 70°C.

[e] Normal tissue is whole rat minus skin, bones, and intestines.

A further attempt was made to modify the activity of a preparation as shown in Table

TABLE II. Plasma Iron Concentration in Rats 16 Hours after an Intraperitoneal Injection of Various Materials.

Material injected[a]	Amt. injected (mg)	No. rats	Plasma iron (μg/100 ml)
Toxohormone preparation	50	17	241 ± 9[b]
Toxohormone preparation after evaporation and resuspension[c]	50	14	130 ± 8
Carbon particles (10–25 mμ diam.)	10	14	155 ± 7
Injection of water only (0.5 ml)	—	14	266 ± 8

[a] Toxohormone preparations were suspended in pyrogen-free water, 100 mg/ml.

[b] Mean ± SE.

[c] The suspended toxohormone preparation (100 mg/ml) was evaporated to dryness in an oven at 105°C and then resuspended in water.

II. Toxohormone fractions suspended in water had only a slight effect upon plasma iron concentration when 50 mg was injected in normal rats. The activity was significantly increased when the suspended fractions of toxohormone were evaporated to dryness in an oven at 105°C and resuspended. Carbon particles of 10–25 mμ diameter were more active than the preparations from either tumor or normal tissues.

Discussion. One of the most likely explanations for lowering of liver catalase activity or decreasing the plasma iron concentration in tumor-bearing animals is that tumor growth initiates toxic effects in the host. Similar toxic effects frequently occur during inflammations or after injecting a wide variety of materials into normal animals (9,18). It would seem unlikely that all of these materials could be acting directly on the target tissue. Thus, there would still seem to be the possibility of an endogenously formed factor both after injections or during tumor growth, similar to endogenous pyrogens (9, 18).

The original concept of toxohormone was that it was synthesized by growing tumor tissue and then transported to its site of action. It is very difficult to obtain positive proof for this concept for the following reasons: The extraction procedures are difficult to standardize; endotoxins are hard to eliminate from tissues such as tumor and to avoid during the long extraction procedures; and the possibilities of autolysis, as pointed out by Olivares *et al.* (13). As indicated by the experiments in this paper, the activity of the extract can be altered by the prior treatment of the tissue, vacuum drying, or by resuspending the final extract. There is also considerable evidence that inert particles such as carbon or talcum (18) are active. These particles are small enough, 10–25 mμ in the present experiments, so that they will remain suspended when centrifuged at 2600*g* for 10 min. This was the centrifugal force used by Hozumi *et al.* (14) to clarify the suspension of crude toxohormone.

Some early experiments by Nakahara and Fukuoka (19) indicated that the biosynthesis of toxohormone would occur under *in vitro* conditions. The amount of synthesis could be controlled by adding the appropriate amino acids. This should provide a method for testing the original toxohormone concept which would eliminate all of the objections listed. Incubation of bacteria-free tumor tissue in the presence and absence of these amino acids should provide a very satisfactory control for subsequent extraction. We were unable to obtain biosynthesis *in vitro* from our tumor tissues in the absence of bacteria (9).

Recently, 3 groups of investigators have found active toxohormone extracts from bacteria-free tumors (8,10,11). None of these experiments, however, fulfilled all of the conditions which now seem necessary to prove the existence of toxohormone. Nakahara *et al.* (11) vacuum dried the germfree tumor tissue at 70°C and control normal tissues were not used. Matsuoka *et al.* (10) also prepared an extract from bacteria-free tumors which would decrease liver catalase activity. They found that similar extracts from bacteria were highly active but suggest that a very heavy contamination with bacteria would be necessary to produce the lowering of liver catalase activity observed with tumor extracts. The extreme variation in activity of their bacteria-free tumor preparations only serves to emphasize that some type of control normal tissue is essential in this type of experiment. In one of their experiments (10), 10 mg of toxohormone lowered catalase 53% while in another the lowering was only 8%. How much of the effect was, therefore, due to autolysis, particle size, etc., rather than a tumor toxin? Nixon and Zinman (8) found an active toxohormone extract from bacteria-free tumors and almost an equal amount from normal kidney or spleen, while other normal tissues had low or no activity.

All of these results illustrate the difficulty involved in showing that these toxic extracts are being synthesized by the tumor tissue. Our findings with bacteria-free tumors are similar to those of the 3 groups listed above in that some depression of both liver catalase activity (7) and plasma iron concentration (Table I) results from extracts prepared from bacteria-free tumors. Since the activity was no greater than some normal tissues prepared at the same time, we interpret our results

as failing to provide evidence for the tumor toxin, toxohormone.

Summary. Extracts of bacteria-free tumor and normal tissues were prepared by methods commonly used to obtain toxohormone and tested by measuring the lowering of plasma iron concentration in rats. The toxohormone extracts of both tumor and normal tissues were active. The plasma iron decrease produced by the extracts could be altered by the methods used in handling the tissue both before extraction and in the final extract. These results provide further evidence that tissue autolysis and procedures employed in extraction and drying can markedly alter the final activity obtained from both tumor and normal tissues. Further proof would, therefore, seem to be required before the synthesis of a tumor toxin, toxohormone, by tumor tissue can be established.

1. Nakahara, W. and Fukuoka, F., Gann **40**, 45 (1949).

2. Nakahara, W. and Fukuoka, F., Advan. Cancer Res. **5**, 157 (1958).

3. Nakahara, W. and Fukuoka, F., "Chemistry of Cancer Toxin, Toxohormone." Thomas, Springfield, Illinois, 1961.

4. Greenfield, R. E. and Meister, A., J. Natl. Cancer Inst. **11**, 997 (1951).

5. Day, E. D., Gabrielson, F. C., and Lipkind, J. B., J. Natl. Cancer Inst. **15**, 239 (1954).

6. Riley, E. E., Cancer Res. **19**, 285 (1959).

7. Kampschmidt, R. F., Schultz, G., Cancer Res. **23**, 751 (1963).

8. Nixon, J. C. and Zinman, B., Can. J. Biochem. **44**, 1069 (1966).

9. Kampschmidt, R. F., Cancer Res. **25**, 34 (1965).

10. Matsuoka, K., Hozumi, M., Koyama, K., Kawachi, T., Nagao, M., and Sugimura, T., Gann, **55**, 411 (1964).

11. Nakahara, W., Hozumi, M., and Pollard, M., Proc. Soc. Exptl. Biol. Med. **123**, 124 (1966).

12. Nakahara, W., Tanaka, T., Tokuzen, R., and Fukuoka, F., Gann **57**, 621 (1967).

13. Olivares, J., Callao, V., and Montoya, E., Science **157**, 327 (1967).

14. Hozumi, M., Ohashi, M., and Ono, T., Gann **56**, 313 (1965).

15. Kampschmidt, R. F., Adams, M. E., and McCoy, T. A., Cancer Res., **19**, 236 (1959).

16. Ono, T., Ohashi, M., and Yago, N., Gann **51**, 213 (1960).

17. Schade, A. L., Oyama, J., Reinhart, R. W., and Miller, J. R., Proc. Soc. Exptl. Biol. Med. **87**, 443 (1954).

18. Nishimura, E. T., Rosenheim, S., and Klein, L., Lab. Invest. **12**, 415 (1963).

19. Nakahara, W. A. and Fukuoka, F. A., Gann **45**, 77 (1954).

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Maturation Changes of Vaginal Epithelium in Pregnant Rats (32761)

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The use of the exfoliative vaginal cytology to evaluate functions of ovaries dates back more than 100 years (6). Later Loch (1) in 1909 described the vaginal epithelium of various animals. Stockard *et al.* in 1917 (2) demonstrated the existence of a typical estrous cycle in guinea pigs, and Allen (4) in 1922, published a monograph entitled "the Oestrus Cycle in the Mouse." The extensive studies of Long and Evans in 1922 (3) on the estrous cycle in rat proved that the cycle is characterized by regular, periodic, coordinated histological changes in the epithelium of the uterus and vagina.

Since no previous work has been done on the maturation changes of vaginal epithelium of the pregnant rat, we have explored this field and submit a report on our observations.

Materials and Methods. Twelve young adult female albino rats of the Walter Reed-Carworth Farm strain were mated. They were placed in four groups of three each. To avoid too frequent handling of the pregnant animals, on day 1 vaginal smears were