2) conversion of palmitoyl alcohol moiety to palmitic acid, and 3) re-esterification of palmitic acid into glycerides and phospholipids must have occurred in the intestinal mucosa. However, unpublished experiments in rats by Blomstrand(23) indicate, that chimyl alcohol can be esterified in the intestinal lumen. This reaction is analogous to the breaking and making of glyceride ester bonds which occurs in the intestinal contents of rats(24) and man (25), presumably under the control of lipase.

Summary. Labelled chimyl alcohol fed orally to a patient with chyluria was almost completely absorbed, and 40% of administered activity was recovered in 12 hours in the urinary lipids. About half of radioactivity in lymph was identified as chimyl alcohol, about three-fourths of which was present as free chimyl alcohol, and a fourth had become esterified. The remaining 50% had been converted to palmitic acid, and found in triglycerides, phospholipids and free fatty acids in proportions expected when dietary palmitic acid is transported from the gut. Repetition of this study in a patient with chylothorax gave essentially the same results. The results indicate that rupture of ether linkage of chimyl alcohol can occur in the intestinal mucosa of man, as in the rat, and that the palmitoyl alcohol moiety is readily oxidized to palmitic acid.

1. Deuel, H. J., Jr., *The Lipids,* N. Y., 1951, vI, 392.
2. ———, *ibid.,* vII, p714.
3. Holman, R. T., Lundberg, W.d., Malkin, T., *Progress in Chemistry of Fats and other Lipids,* N. Y., v5, p5.
4. Hardegger, E., Ruzicka, L., Tagman, E., *Helv. Chim. Acta,* 1943, v26, 2295.
5. Lovern, J. A., *Lipids of Biochemical Significance,* v. p31.
6. Holmes, N. N., Corbet, R. E., Geiger, W. B., Kornblum, N., Alexander, W., *J. Am. Chem. Soc.,* 1941, v63, 2607.
7. Marberg, G. M., Wiles, O. H., *Arch. Int. Med.,* 1938, v61, 408.
8. Sandler, O. E., *Acta Med., Scand. Suppl.,* 1949, 225.
9. Edlund, T., *Nature,* 1954, v174, 1102.
10. Brohult, A., Holmberg, J., *ibid.,* 1954, v174, 1102.
11. Evans, W. C., Evans, I. A., Edwards, C. H., Thomas, A. J., *Biochem. J.,* 1957, v65, 6p.
12. Schultze, M. O., Perman, V., Bates, F. W., Sautter, J. H., PROC. SOC. EXP. BIOL. AND MED., 1958, v98, 470.
13. Bergström, S., Blomstrand, R., *Acta Physiol. Scand.,* 1956, 38, 166.
14. Blomstrand, R., Thorn, N., Ahrens, E. H., Jr., *Am. J. Med.,* 1958, v24, 958.
15. Blomstrand, R., Ahrens, E. H., Jr., *J. Biol. Chem.,* 1958, v233, 621.
16. ———, *ibid.,* v958, v233, 627.
17. Linder, E., Blomstrand, R., PROC. SOC. EXP. BIOL. AND MED., 1958, v97, 653.
18. Brown, W. D., *Science,* 1953, v118, 46.
19. Borgström, B., *Acta Physiol. Scand.,* 1954, v30, 231.
20. Hirsch, J., Ahrens, E. H., Jr., *J. Biol. Chem.,* 1958, v233, 311.
21. Hilditch, T. P., Lea, C. H., *J. Chem. Soc.,* 1927, 3106.
22. Howard, G. H., Martin, H. J. P., *Biochem. J.,* 1950, v46, 532.
23. Blomstrand, R., *Acta Physiol. Scand.,*
24. Borgström, B., *Arch. Biochem.,* 1954, v49, 268.
25. Ahrens, E. H., Borgström, B., *J. Biol. Chem.,* 1956, v219, 665.

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Growth of Human Cancer (H Ep 3) in Normal Rats.* (24784)

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Many workers have attempted to transplant human cancers into experimental animals but only a few have devised successful procedures. Greene(1,2), by inoculating into the anterior chamber of the eye, or into the brain of normal animals found that tumors "in the final developmental stages of meta-

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stasizability" would continue to grow in these sites. Chute *et al.*(3), implanted human cancer into the membranous wall of the cheek pouch of hamsters and obtained growth of about half of the specimens investigated. Toolan(4) demonstrated that some cancers would grow when implanted subcutaneously or in other sites of animals that had been treated with X-irradiation and/or cortisone. These cancers could be propagated in series, in animals so treated, but failed to grow in normal animals. The consensus is that failure of cancer transplants to grow in subcutaneous tissue of normal animals is due to an immunologic response on the part of recipient animal to antigens in implanted tissue. It occurred to us that one might circumvent this immunologic rejection of the cancer by taking advantage of the phenomenon of immunologic tolerance first described by Billingham *et al.* (5). Using Toolan's H Ep 3 cancer and inoculating it directly into fetal rats, we have shown that it will continue to grow in these animals after birth, frequently causing their death.

Materials and methods. The H Ep 3 was obtained from Sloan-Kettering Institute for Cancer Research through the courtesy of Drs. Toolan and Teller. We have maintained it as "stock" tumor in our laboratory by serial passage in weanling Wistar rats treated with 150 r of X-irradiation and 4 subsequent injections of 3 to 6 mg of cortisone acetate given on alternate days. Transplants have been made at 7 to 10 day intervals. Malignant tissue removed from stock animals was minced with scissors and ground to fine suspension in a sterile mortar with either Hanks' or Eagle's balanced salt solution as the suspending medium. For stock passage in weanling rats the suspension was drawn up through a 20 gauge hypodermic needle and inoculated subcutaneously in 0.5 ml amounts. For inoculation into fetal animals the suspension was drawn up through a 27 gauge needle and 0.02 to 0.03 ml was injected into each fetus. Pregnant rats were of Wistar strain obtained from Carworth Farms and bred in our laboratories. Pregnancies were timed to within 24 hours. The technic of fetal inoculation was that devised by Woolpert(6) and used previously by one of us (F.W.G.)(7) for propagation of viral agents in fetal animals. Employing an apparatus described by Woolpert(8) for continuous administration of ether, and using aseptic precautions, a mid-line abdominal incision was made. Horns of gravid uterus were brought out and the fetuses, clearly visible through uterine wall, were inoculated (care being taken to avoid trauma to the central nervous system) into the thoracic cavity, peritoneal cavity or subcutaneous tissue. To minimize deleterious effects of anesthetic on the fetuses, rate of administration of ether was slowed and finally stopped during suturing of incision, so that when operation was complete the animal was nearly awake. When animal had given birth to her young she was disturbed as little as possible for 24 hours after which the young were examined daily for evidence of cancerous growths. All animals either died spontaneously or were sacrificed. They were dissected and examined and appropriate pieces of tissue were taken for histologic examination.

Results. Animals tolerated inoculation procedure very well; 85% of inoculated fetuses were born alive. This contrasts with results of Egdahl *et al.*(9) who, in attempting to produce heterologous tolerance in rats by inoculating rat fetuses with rabbit or mouse cells, encountered 60% to 80% fetal mortality.

Of animals born alive, a high percent had malignant growths; in several instances every animal in a litter developed cancer. The tumors grew rapidly and often attained a considerable size, sometimes reaching a diameter of 2 cm. These growths frequently led to death of animals, usually within 2 weeks after inoculation. Tumors in thoracic or peritoneal cavities killed more quickly and more regularly than did subcutaneous growths. In a few instances rapidly growing subcutaneous tumors began to regress about 2 weeks after inoculation and finally disappeared.

Serial passage of the cancer in these normal animals has been achieved readily. Tissue taken from young rats that had been inoculated while *in utero,* was reinoculated into new litters of fetuses. At this time fetal passage cancer is in the fourth serial passage and has retained its original characteristics. When in-

oculated into weanling rats (treated with X-irradiation and cortisone) it produced tumors identical to stock tumors in gross and microscopic appearance, and failed to grow when inoculated into untreated weanling rats. Microscopic examination of fetal passage tumors showed that they were identical to original stock cancer.

It is not possible to state definitely the most appropriate fetal age for inoculation of H Ep 3 cancer. We have used fetuses of 19, 20, 21 and 22 days gestation age, and one litter of newborn rats (less than 12 hours old). None of the newborn rats developed cancer; only one rat in a litter inoculated on 22nd gestation day (*i.e.*, 6 hours before birth) developed a cancer, whereas most animals inoculated at 19 to 20 days gestation age developed cancers. To obtain more definitive data we are repeating these experiments using animals whose pregnancies are timed to within 8 hours. However, from results to date it seems reasonable to infer that susceptibility of the normal rat to H Ep 3 cancer is lost about the time of birth.

Discussion. We believe that this is the first time a human cancer has been propagated in series in subcutaneous tissue of normal mammals. There can be no doubt that H Ep 3 is a human cancer, for Korngold(10) has demonstrated clearly that it retains its human antigenicity. Using similar methods Koprowski *et al.*(11) and Wallace(12) were able to grow rat and mouse tumors in fetuses of resistant strains of the animals. Wallace(12) was unsuccessful however in propagating human cancer cell line HeLa in fetal rats. Dagg, Karnofsky and Roddy(13) grew H Ep 3 in normal chicks by inoculating them either as embryos or as newly hatched (up to 3 days) birds.

There occur 2 possible explanations as to why cancer grows so readily following inoculation into the fetus. One is that the inoculum has induced in these animals a specific immune tolerance to foreign antigens of the cancer. Some doubt, however, is cast on the validity of this explanation, because of the regression of some tumors growing subcutaneously. This suggests development by the animals of immunity against the cancer and we have under way experiments designed to evaluate their immune status.

The second explanation is that the tumor has a high growth potential and, by taking advantage of increased susceptibility of the fetus it "gets a running start" which enables it to keep ahead of the resistance mechanism which first appears about time of birth. If this be true then neoplasms with slower growth rates might be expected to behave differently from fast growing H Ep 3.

Summary. It has been demonstrated that human cancer H Ep 3 when inoculated into fetal rats a few days before birth, proliferated in these animals and continued to do so after birth. The cancers grew to large size and when located in the thoracic or peritoneal cavities usually killed the animals within 2 weeks. Serial passage of tumor by fetal inoculation was achieved readily and obviated treatment of animals with X-irradiation and/or cortisone. The cancer retained its original characteristics after fetal passage.

1. Greene, Harry S. N., *J. Exp. Med.*, 1941, v73, 475.

2. ———, *Cancer Research,* 1951, v11, 529.

3. Chute, R. N., Sommers, S. C., Warren, S., *ibid.*, 1952, v12, 912.

4. Toolan, H. W., *ibid.*, 1954, v14, 660.

5. Billingham, R. E., Brent, L., Medawar, P. B., *Nature,* 1953, v172, 603.

6. Woolpert, O. C., *Am. J. Path.,* 1936, v12, 141.

7. Gallagher, F. W., *Am. J. Pub. Health,* 1955, v45, 885.

8. Woolpert, O. C., *J. Lab. and Clin. Med.,* 1936, v22, 298.

9. Egdahl, R. H., Roller, F. R., Swanson, R. L., Varco, R. L., *Ann. N. Y. Acad. Sci.,* 1958, v73, 843.

10. Korngold, L., Lipari, R., *Cancer Res.,* 1955, v15, 159.

11. Koprowski, H., Theis, G., Love, R., *Proc. Roy. Soc.,* 1956, v146B, 37.

12. Wallace, A. C., *Cancer Res.,* 1956, v16, 348.

13. Dagg, C. P., Karnofsky, D. A., Roddy, J., *ibid.*, 1956, v16, 589.

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