Summary. The presence in human erythrocyte stroma of a chromogenic substance giving absorption spectra (direct Ehrlich and Bial reactions) identical with those of crystalline sialic acid has been confirmed. A potent water-soluble inhibitor of viral hemagglutination, containing 7-11% by weight of this chromogen, has been isolated by partition dialysis from concentrated human erythrocyte stroma. 30-40% of the chromogen in the crude stroma concentrate and up to 56% of that in the purified inhibitor was rendered dialyzable following treatment with concentrated infective influenza virus, which in most instances also destroyed receptors for indicator viruses. Limited chromatographic analysis indicated that the dialyzable chromogen is most probably sialic acid. These observations represent the first direct demonstration of split products resulting from enzymic action of influenza virus on human erythrocyte components.

1. Blix, F. G., *Z. f. Physiol. Chem.*, 1936, v43, 240.
2. Gottschalk, A., *Yale J. Biol. and Med.*, 1956, v28, 525.
3. Heimer, R., and Meyer, K., *Proc. Nat. Acad. Sci.*, 1956, v42, 728.
4. Gottschalk, A., *Nature*, 1951, v167, 845.
5. Howe, C., *J. Immunol.*, 1951, v66, 9.
6. ———, *Fed. Proc.*, 1957, v16, 418.
7. Markham, R., *Biochem. J.*, 1942, v36, 790.
8. Elson, L. A., and Morgan, W. T. J., *ibid.*, 1933, v27, 1824.
9. Hagedorn, H. C., and Jensen, B. N., *Biochem. Z.*, 1923, v135, 46.
10. King, E. J., *Biochem. J.*, 1932, v26, 292.
11. Werner, I., and Odin, L., *Acta Soc. Med. Upsaliensis*, 1952, v57, 230.
12. Rosenberg, A., Howe, C., and Chargaff, E., *Nature*, 1956, v177, 234.
13. Marmion, B. P., Curtain C. C., and Pye, J., *Australian J. Exp. Biol. and Med. Sci.*, 1953, v31, 505.
14. Burnet, F. M., and Stone, J. D., *ibid.*, 1947, v25, 227.
15. Folch, J., Arsove, S., and Meath, J. A., *J. Biol. Chem.*, 1951, v191, 819.
16. Klevstrand, R., and Nordal, A., *Acta Chem. Scand.*, 1950, v4, 1320.
17. Magasanik, B., Vischer, E., Doniger, R., Elson, D., and Chargaff, E., *J. Biol. Chem.*, 1950, v186, 37.
18. Kuhn, R., and Brossmer, R., *Chem. Ber.*, 1954, v87, 123.
19. Klenk, E., and Lempfrid, H., *Z. f. Physiol. Chem.*, 1957, v307, 278.
20. Blix, F. G., Gottschalk, A., and Klenk, E., *Nature*, 1957, v179, 1088.
21. Howe, C., MacLennan, J. D., Mandl, I., and Kabat, E. A., *J. Bact.*, in press.
22. deBurgh, P. M., Yu, P., Howe, C., and Bovarnick, M., *J. Exp. Med.*, 1948, v87, 1.
23. McCrea, J. F., *Yale J. Biol. and Med.*, 1953, v26, 191.

Received July 3, 1957. P.S.E.B.M., 1957, v96.

Growth of Human Tumors in Hibernating Hamsters.* (23402)

W. BRADFORD PATTERSON,‡ CHARLES P. LYMAN AND HELEN R. PATTERSON
(Introduced by R. O. Greep)
Departments of Surgery and Anatomy, Harvard Medical School; and the Department of Surgery Peter Bent Brigham Hospital

Previous studies by Lyman and Fawcett (1) have shown that the growth rate of homologous tumor transplants in the hamster cheek pouch was diminished when the animals were induced to hibernate. The effect of the low temperature of hibernation (5°C) on the tissue of an animal which does not hibernate has not been previously tested. Lyman and Fawcett suggested that such a tissue might be less resistant to cold since there are apparently innate differences between the cellular physiology of hibernating and non-hibernating mammals. With the recent development of technics for transplanting human tumors

* This research was supported in part by a grant from the American Cancer Society, and in part by Air Force Contract 33(038)-18133.

‡ Present address: Sears Surgical Laboratory and Fifth (Harvard) Surgical Service, Boston City Hospital, 818 Harrison Avenue, Boston, Mass.

to the cheek pouch of cortisone-treated hamsters(2,3) it has become possible to test the response of human tissues under the conditions of hibernation. During the past year we have tested 5 transplantable human tumors. Our results indicate that the growth of this tissue from a homeothermic mammal is markedly inhibited by hibernation, but that the tumor survives after prolonged exposure to these low temperatures. The possibility that the growth rate of these heterologous tumors might be slowed down sufficiently to provide a means of temporarily "storing" transplantable human tumors was our primary reason for carrying out this study.

Materials and methods. The tumors used in this study had been transplanted through many generations of hamsters over periods of 2 to 3 years(4), and with one exception were first transplanted in this laboratory from surgical specimens. The Pitt-41 strain was originally obtained from an autopsy specimen at the University of Pittsburgh.

Hamsters, 5 to 10 months of age, were kept in a cold room at 5° ± 2°C until they hibernated (see Lyman(5) for details). Tumor fragments one to 2 mg in size were then implanted in the submucosal space of each cheek pouch by means of trocar injection. In some animals a 50 mg pellet of cortisone† was implanted subcutaneously at this time, in others 2.5 mg of cortisone acetate suspension was injected by the same route. The latter group were given additional doses of 2.5 mg approximately twice a week according to requirements previously worked out for different tumor strains. The implantation technics invariably awakened the hibernating animals, but most of them soon returned to hibernation. The hamsters were checked daily thereafter and a light sprinkling of sawdust was dusted on the backs of the hibernating animals. If the sawdust were absent the next day, but the animal in hibernation, it was assumed that the animal had awakened and returned to hibernation. In calculating the total days in hibernation, such an animal was arbitrarily considered to have hibernated one-half a day. Tumors were inspected at intervals but no attempt was made to construct growth curves, since the usual pattern of growth at room temperature is quite predictable and our interest lay in the delay of growth during hibernation.

The hamsters were kept in the cold for 4 to 6 weeks and then moved to the warm room (23°C) where observations were continued until the tumors had either grown to the usual upper limits in size or obviously regressed. The animals were then sacrificed and the histology of all growing tumors confirmed by biopsy. A few transplants were made from these tumors to demonstrate that further propagation was feasible.

Results. The tumor strains tested will normally grow in at least three-fourths of the animals to nodules weighing 100 to 1000 mg (0.5 cm to 1.5 cm diameter). The growth period is 3 to 8 weeks; regression invariably occurs thereafter and metastases are not seen. The usual growth and the effect of hibernation upon it are reported below for each of the 5 strains.

(a) *Deac*—1 (Epidermoid carcinoma of the parotid gland). This tumor ordinarily grows to a diameter of 0.4 to 0.8 cm in 18 to 24 days and regresses completely in 30 to 42 days. In 2 test animals which hibernated more than 50% of the time, tumors grew only to 0.1 to 0.3 cm during a 4-week period in the cold. After 4 additional weeks at 23°C these animals had healthy tumors 0.42 to 0.73 cm in diameter.

(b) *Deac*—3 (Embryonal carcinoma of the testis). This ordinarily grows to a diameter of 0.8 to 1.2 cm in 21 to 28 days and begins to regress a few days later. Four test animals hibernated 49 to 74% of the 5 weeks, during which time no tumor grew beyond 0.25 cm in diameter although all were obviously vascularized. After the subsequent 3 weeks in the warm room, half of the tumors grew to average size.

(c) *Deac*—6 (Adenoacanthoma of the endometrium). This tumor reaches a diameter of 0.5 to 0.7 cm in 4 to 6 weeks with degeneration occurring soon thereafter. Typical normal growth to 0.45 cm in diameter was observed in one animal which hibernated only 2

† "Cortagen", furnished to us through the courtesy of the Schering Corp.

days of the 5 weeks in the cold. Three other animals hibernated 64 to 76% of the 5 week period. The largest tumor at the end of this time was only 0.2 cm in diameter, but during the subsequent 3 weeks in the warm room all grew to the usual size.

(d) *Deac*—8 (Adenocarcinoma of the endometrium). The usual period of growth for this tumor is 5 to 7 weeks with the tumor averaging 0.5 to 0.9 cm in diameter at the end of this period. The 4 test animals hibernated 43 to 72% of the 5 week period and no growth was apparent during this time, nor did growth occur during the ensuing 3 weeks in the warm room. The reason for this failure is not known. It is possible that this particular tumor tissue cannot tolerate such an exposure to cold, but more likely that it failed because of all our tumors it is one of the most slowly growing and difficult to maintain.

(e) *Pitt*—41 (Undifferentiated carcinoma of thyroid origin). The average period of growth is 14 to 21 days, with tumors reaching 0.9 to 1.5 cm in diameter. Five test animals hibernated 60 to 74% of the 5 weeks. Tumor growth was markedly delayed, with diameters averaging only 0.4 cm at the end of this period. Further transplants made from these tumors at this time grew successfully at the normal rate.

Discussion. Previous studies have shown that certain tissues from mammals which hibernate continue overt function at much lower temperatures than do similar tissues from non-hibernators(6,7). For example, an action potential can be elicited from the tibial nerve of the hamster *in vitro* at an average temperature of 3.4°C, while the same nerve in the rat ceases to function at about 9°C. This characteristic appears to be an innate difference of the animals in question, for there is no evidence that the ventral caudal nerve of the rat adapts to either high or low temperatures(8). Furthermore, it has been known for some time that mammals which hibernate can survive chilling to much lower temperatures than can non-hibernating mammals(9). Thus the hamster will recover from a deep body temperature of 3.8°C(10) while 18°C seems to be about the minimum temperature which the human can tolerate(11).

Although no quantitative comparison may be made with such a small sample, the heterologous tumors investigated in this study appeared to be less viable during hibernation than were the homologous tumors tested previously(1). However, most of the human tumors remained alive for many days in the cheek pouches of hibernating hamsters whose body temperatures are known to be less than 1°C above the environmental temperature of 5°C (12). Unless the host in some way changes the temperature tolerance of the implant, it would appear that human tumor tissue will survive well below the temperature where human tissue ceases all observable function.

As a means for "cold storage" of human tumor tissue, this technic is probably feasible. Rapidly growing strains which ordinarily require transplantation every 10 to 14 days could be transplanted only a third as often in animals maintained in the cold room. However, tumor storage in the frozen state is possible with many animal tumors and may prove more suitable for human material. Haemmerli and Toolan(13) have reported success in freezing a number of human transplantable strains for periods up to 6 months. We are investigating this problem at present and have had similar results.

Summary. Four human transplantable tumors have been grown in hibernating hamsters. One human tumor strain failed to grow. During hibernation tumor growth was markedly inhibited, but resumed its normal rate when animals were returned to warm room temperatures.

1. Lyman, C. P., and Fawcett, D. W., *Cancer Research*, 1954, v14, 25.

2. Lutz, B. R., Fulton, G. P., Patt, D. I., and Handler, A. H., *ibid.*, 1950, v10, 231.

3. Patterson, W. B., *Surg. Forum*, 1954, v5, 637.

4. Patterson, W. B., Patterson, H. R., and Chute, R. N., *Cancer*, in press.

5. Lyman, C. P., *J. Mammal.*, 1954, v35, 545.

6. Tait, J., *Am. J. Physiol.*, 1922, v59, 467.

7. Chatfield, P. O., Battista, A. F., Lyman, C. P., and Garcia, J. P., *ibid.*, 1948, v155, 179.

8. Chatfield, P. O., and Lyman, C. P., *ibid.*, 1954, v177, 183.

9. Horvath, A., *Verhandl. d. phys.-med. Gesellsch.*, 1881, v15, 187.

10. Adolph, E. F., and Lawrow, J. W., *Am. J. Physiol.*, 1951, v166, 62.
11. Laufman, H., *J.A.M.A.*, 1951, v147, 1201.
12. Lyman, C. P., *J. Exp. Zool.*, 1948, v109, 55.
13. Haemmerli, G. W., and Toolan, H. W., *Proc. Am. Assn. Cancer Res.*, 1957, v2, 209.

Received July 8, 1957. P.S.E.B.M., 1957, v96.

Disappearance of Radioactive Iron from Plasma by Day and by Night. (23403)

J. C. S. PATERSON (Introduced by Grace A. Goldsmith)
Department of Medicine, Tulane University School of Medicine, New Orleans, La.

It has been shown that the diurnal variation of the plasma iron level is less evident in aplastic anemia, pernicious anemia in relapse, and in iron-deficiency anemia, and that full treatment of the 2 last conditions restores a normal diurnal variation. The diurnal variation was also found to be diminished and the plasma iron levels low in various conditions associated with increased erythropoiesis; polycythemia vera, congenital hemolytic anemia, nocturnal hemoglobinuria, pernicious anemia at the height of the reticulocyte response, iron-deficiency anemia treated with a limited quantity of iron(1) and in sickle-cell anemia (2). In similar conditions of increased erythropoietic activity, Huff *et al.*(3) found an increased plasma iron turnover. Under these circumstances the plasma iron level is low and remains relatively constant throughout the 24 hours. There is little or no rise in the level during sleep, possibly because the bone marrow is actively taking up iron during sleep. If this postulate be true it is reasonable to infer that the normal diurnal variation is brought about by diminished bone marrow activity during sleep and hence an increasing plasma iron level. In this respect it was especially interesting to find that the plasma iron level remained low throughout the 24 hours in the 2 patients with paroxysmal nocturnal hemoglobinuria, the hemolytic process in each case being compensated by increased erythropoietic activity.

Laurell(4) on the other hand noted the similarity between the diurnal plasma iron and bilirubin levels and explained both on the assumption that rate of destruction of hemoglobin is slightly greater during the night than during the day. It therefore seemed important to determine the fate of radioactive iron injected into the plasma by day and by night.

Method. The method adopted was similar to that of Wasserman *et al.*(5) except that the radioactive iron was converted to siderophilin by addition to the individual's plasma *in vitro*(6) and not by addition to Fraction IV-7 protein. Radioactive iron (^{59}Fe), in the form of ferric chloride, was neutralized with sterile NaOH and added to the plasma which was then allowed to stand for several hours before injection into the blood stream. After injection of radioactive iron, blood samples were collected by means of an in-dwelling needle in an antecubital vein and the subjects (except Subject 1) slept comfortably during the night experiments. Observations were made by day and by night (within a 24-hour period) on 3 normal individuals, one patient suffering from iron deficiency anemia and one from sickle-cell anemia. Diurnal plasma iron levels were determined by methods described previously(7).

Values for plasma iron turnover and the fraction of radio-iron removed per hour were calculated as in the paper by Huff *et al.*(3).

Results. For the 3 normal individuals T½ by day was found to be 96, 101 and 88 minutes and T½ by night 128, 126 and 126 minutes, respectively. The time-courses for Subject No. 3 are shown graphically in Fig. 1. In the patient with iron-deficiency anemia the clearance rates were almost identical by day and by night. Only one curve could be fitted, and T½ by day and by night was found to be 56 minutes (Fig. 2). In the patient with sickle-cell anemia the clearance rates were