a depression of PAH secretion and/or a decrease in renal blood flow.

1. Barac, G., *C. R. Soc. Biol.,* 1959, v153, 704.
2. Gross, F., Turrian, H., in M. Schachter, Ed., *Polypeptides Which Affect Smooth Muscles and Blood Vessels,* Oxford, Pergamon Press, 1960, p137.
3. Del Greco, F., Page, I. H., *Circulation,* 1961, v24, 917.
4. Finnerty, F. A., Jr., *ibid.,* 1962, v25, 255.
5. Bock, K. D., Dengler, H., Krecke, H. J., Reichel, G., *Klin. Wochenschr.,* 1958, v36, 808.
6. Peart, W. S., *Brit. Med. J.,* 1959, vII, 1421.
7. Mandel, M. J., Sapirstein, L. A., *Circulation Res.,* 1962, v10, 807.
8. McGiff, J. C., Aviado, D. M., *ibid.,* 1961, v9, 1327.
9. Peart, W. S., Brown, J. J., *Lancet,* 1961, vI, 28.
10. Barbour, B. H., Gill, J. R., Jr., Slater, J. D. H., Bartter, F. C., *Clin. Res.,* 1962, v10, 245.
11. Brown, J. J., Peart, W. S., *Clin. Sci.,* 1962, v22, 1.
12. Del Greco, F., PROC. SOC. EXP. BIOL. AND MED., 1961, v107, 943.
13. Biron, P., Chrétien, M., Kolw, E., Genest, J., *Brit. Med. J.,* 1962, vI, 1568.
14. Cotlove, E., *J. Appl. Physiol.,* 1961, v16, 764.
15. Peters, G., Hedwall, P. R., *Arch. int. pharmacodyn. et thér.,* in press, 1962.
16. Peters, G., *Arch. exp. Path. u. Pharmakol.,* 1958, v235, 113.
17. Gross, F., Bock, K. D., *Circulation,* 1962, v25, 193.
18. Croxatto, H., Barnafi, L., Passi, J., *Acta physiol. latino-amer.,* 1952, v2, 159.
19. Schroeder, R., Meyer-Burgdorff, C., Rott, D., Brahms, O., *Arch. exp. Path. u. Pharmakol.,* 1962, v240, 285.
20. Brunner, H., Regoli, D., *Experientia,* 1962, v18, 504.
21. Goll, F., *Z. f. rat. Med.,* 1854, vIV, 86.
22. Selkurt, E. E., *Circulation,* 1951, v4, 541.
23. Thurau, K., Deetjen, P., *Pflüger's Arch.,* 1962, v274, 567.
24. Leyssac, P., Lassen, U. V., Thaysen, J. H., *Biochim. biophys. acta,* 1961, v48, 602.
25. Burg, M. B., Orloff, J., *Am. J. Physiol.,* 1962, v202, 565.
26. Hughes-Jones, N. C., Pickering, G. W., Sanderson, P. H., Scarborough, H., Van Den Broucke, J., *J. Physiol.,* 1949, v109, 288.
27. Carpenter, C. C. J., Davis, J. O., Ayers, C. R., *J. Clin. Invest.,* 1961, v40, 2026.
28. Davis, J. O., Ayers, C. R., Carpenter, C. C. J., *ibid.,* 1961, v40, 1466.
29. Ganong, W. F., Mulrow, P. J., Boryczka, A., Cera, G., PROC. SOC. EXP. BIOL. AND MED., 1962, v109, 381.
30. Mulrow, P. J., Ganong, W. F., *Circulation,* 1962, v25, 213.
31. Mulrow, P. J., Ganong, W. F., Cera, G., Kuljian, A., *J. Clin. Invest.,* 1962, v41, 505.
32. Gláz, E., Sugar, K., *J. Endocrinol.,* 1962, v24, 299.
33. Peters, G., *Arch. exp. Path. u. Pharmakol.,* 1958, v235, 155.
34. Del Greco, F., PROC. SOC. EXP. BIOL. AND MED., 1961, v107, 943.

Received January 11, 1963. P.S.E.B.M., 1963, v112.

Colchicine Teratogenesis in Hamster Embryos.* (28167)

VERGIL H. FERM (Introduced by R. Gosselin)
Department of Pathology, Dartmouth Medical School, Hanover, N. H.

The natural resistance of the adult golden hamster, *Cricetus auratus,* to the mitotic inhibitory action of colchicine has been described(1) and confirmed(2,3). It has also been reported that hamster embryonic cells grown in tissue culture have at least a 100-fold greater resistance to colchicine than do human cells in tissue culture(3). However, Vincaleukoblastine, another mitotic inhibitor, has as marked an effect on hamsters as on other animals(4). During a study on mitotic inhibition in hamster embryos it was noted that colchicine had an unexpected effect on the developing embryos. This report is based on a study of the effects of colchicine in pregnant hamsters, using fetal mortality and congenital malformations as criteria.

Materials and methods. Virgin female golden hamsters were bred under direct observation in the early evening hours and left with the male overnight. The day following

* Supported by U.S.P.H.S. grant.

TABLE I. Effect of Colchicine on Fetal Survival and Gross Congenital Malformations when Injected Intravenously into Golden Hamsters on 8th Day of Gestation.

Dosage of colchicine (mg/kg)	No. of litters	Fetuses treated	Fetuses surviving	% mortality	No. of survivors grossly abnormal
10	7	84	41	51	9
20	4	50	2	96	2
50	1	14	0	100	–
Controls	10	119	8	7	0

the evening of breeding was considered to be the first day of gestation. Preliminary experiments in this laboratory have shown that pregnant hamsters treated with large doses of Vit. A on the eighth day of gestation produced a wide spectrum of anomalies in the young. This day of gestation was, therefore, selected for treatment with colchicine. Colchicine was prepared as a 0.40% solution in normal saline and, under Nembutal anesthesia, various dosages of colchicine (Table I) were injected directly into the femoral vein after it had been exposed by a small cutaneous incision. The animals were maintained on Purina lab chow and water until the fourteenth day of gestation when they were sacrificed, the fetuses recovered and fixed in either Bouin's fixative for histological studies or in alcohol-formalin-acetic acid for gross examination and photography. Certain selected fetuses were fixed in alcohol, cleared in 1% KOH and the skeletons stained with Alizarin Red according to the Dawson method (5). Control animals were similarly injected with similar or greater volumes of normal saline.

Results. The results are summarized in Table I. Colchicine in dosages of 10 mg/kg on the 8th day of gestation produced a 50% mortality of hamster embryos, as calculated by adding the number of survivors and resorption sites at time of sacrifice on day 14. In the survivors, colchicine also produced a number of gross congenital malformations consisting of microphthalmia and anophthalmia, both unilateral and bilateral, exencephaly of various degrees, umbilical hernia, and skeletal malformations, mainly fused ribs (Fig. 1-6). One or more malformed embryos were found in each litter of those hamsters treated on the 8th day with 10 mg/kg and 20 mg/kg of colchicine. Histological examination of maternal, placental and fetal tissues taken at time of sacrifice revealed no abnormal patterns of mitotic activity. Maternal kidneys were normal.

Increasing the amount of colchicine increased fetal mortality precipitously. Those few fetuses which survived an injection of 20 mg/kg showed evidence of anophthalmia. Maternal animals did not appear to be affected by the treatment, exhibiting normal weight gains if they were carrying viable fetuses, but failing to gain weight if all fetuses were found to be resorbed. There was no evidence of diarrhea, hematuria, or anorexia in any of the maternal animals.

Discussion. The marked colchicine resistance of adult and embryonic hamster tissue would seem to make it unlikely that this compound would have a teratogenic effect *in vivo*. Nevertheless, the high fetal mortality rate and the occurrence of at least one or more congenitally malformed embryos in 10 treated litters makes it quite apparent that colchicine has a definite effect on hamster embryonic tissue *in vivo*. It is most probable that the embryocidal and teratogenic effect of colchicine was exerted on the 8th day of gestation, within a few hours after injection, since few malformations of the type encountered here can be induced in the hamster after this day of gestation(6). The increased resistance of hamster embryonic cells to colchicine reported by Midgely *et al.*(3), may not be present or so marked in embryonic cells on the 8th day of gestation *in utero*. The rather wide variety of malformations induced by colchicine in the hamster suggests that a number of embryonic cells must be susceptible to its effects. More careful search by serial sections might well show other malformations, some detectable

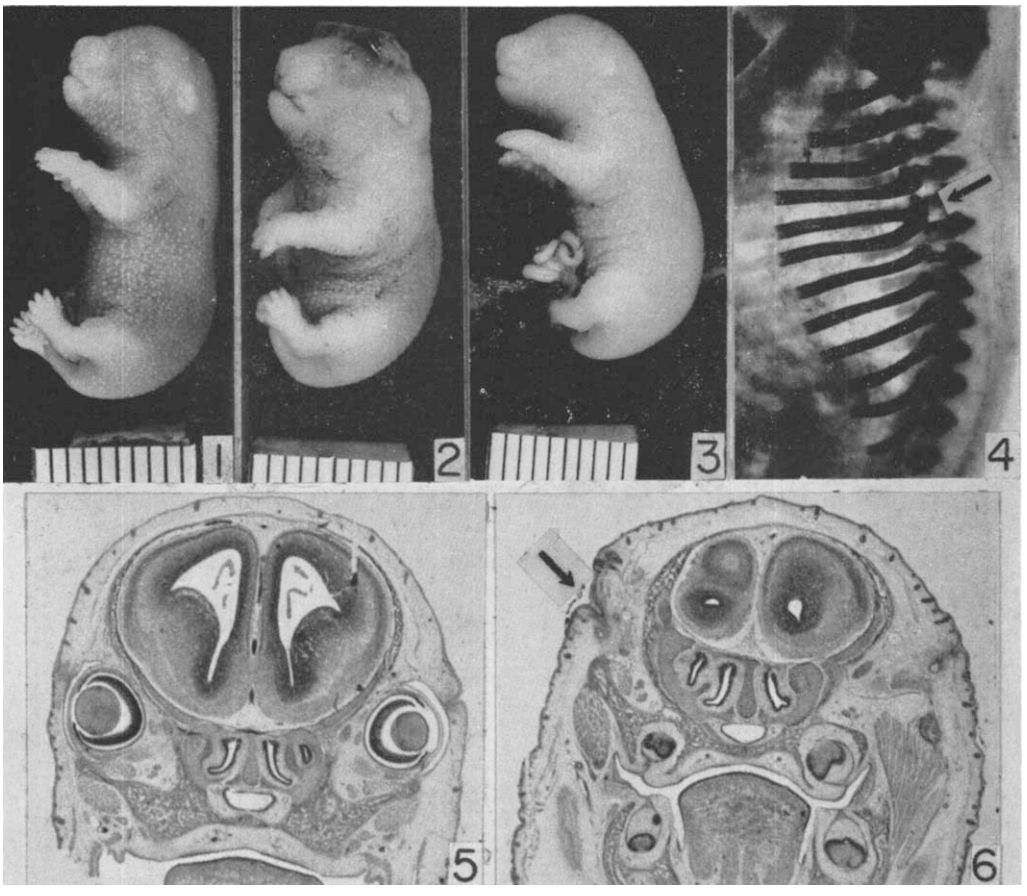

FIG. 1. Normal 14-day hamster fetus from control series. ×2.
FIG. 2. Exencephaly in 14-day hamster fetus treated with 10 mg/kg colchicine on day 8. ×2.
FIG. 3. Umbilical hernia in 14-day hamster fetus treated with 10 mg/kg colchicine on day 8. ×2.
FIG. 4. Alizarin Red-stained vertebral column and rib cage of 14-day hamster fetus showing rib fusion (arrow). Treated with 10 mg/kg colchicine on day 8. ×7.
FIG. 5. Cross section of normal 14-day hamster fetus through eyes. ×11.
FIG. 6. Bilateral anophthalmia in 14-day hamster fetus treated with 10 mg/kg colchicine on day 8. Evidence of eyelid formation only (arrow). ×10.

only by microscopic means.

The fact that the maternal animals did not show evident symptoms from the colchicine treatment agrees with findings in other hamster teratogenic experiments in which the experimental method had no effect on the maternal system(7). Dosages of colchicine in the range of 10 mg/kg, as used in this experiment, have been reported to show a somewhat increased mitotic index in bone marrow cells (4) but not in cells of the intestinal crypts of adult hamsters(3).

It is possible that the teratogenic effect of colchicine may not be related to its effect on cell mitosis but to some other pharmacological action, such as its effect on the suppression of acute gout if, indeed, this eventually proves to be other than a mitosis-inhibiting effect. In rabbits, however, colchicine had a teratogenic effect when spermatozoa were treated prior to artificial insemination(8), and derivatives of this drug have a suppressive effect on blastocyst growth when injected early in gestation(9). Further studies to determine the permeability of the placenta to colchicine and similar compounds are now in progress.

Summary. Colchicine injected intrave-

nously into pregnant hamsters on the 8th day of gestation at levels of 10 mg/kg produces a fetal mortality of 50%. Gross congenital malformations, consisting of microphthalmia, anophthalmia, umbilical hernia, exencephaly, and skeletal anomalies are found in a significant number of survivors.

I wish to acknowledge the technical assistance of Miss Peggy Starusky and Mrs. Jane Juergens.

1. Orsini, M. W., Pansky, B., *Science,* 1952, v115, 88.

2. Turbyfill, C. L., Soderwall, A. L., *ibid.,* 1957, v126, 749.

3. Midgley, A. R., Pierce, B., Dixon, F. J., *ibid.,* 1959, v130, 40.

4. Cardinali, G., Cardinali, G., Handler, A. H., Agrifoglio, M. F., PROC. SOC. EXP. BIOL. AND MED., 1961, v107, 891.

5. Dawson, A. B., *Stain Technol.,* 1926, v1, 123.

6. Harvey, E. B., Chang, M. C., *J. Cell. & Comp. Physiol.,* 1962, v59, 293.

7. Ferm, V. H., *J. Embryol. exp. Morph.,* 1958, v6, 284.

8. Chang, M. C., *Nature,* 1944, v154, 150.

9. Adams, C. E., Hay, M. F., Lutwak-Mann, C., *J. Embryol. exp. Morph.,* 1961, v9, 468.

Received January 14, 1963. P.S.E.B.M., 1963, v112.

Insulin Binding Proteins in Human Serum. *In vitro* Experiments. (28168)

JØRGEN CLAUSEN, FLEMMING GJEDDE AND KLAVS JØRGENSEN
(Introduced by N. A. Thorn)
Departments of Biochemistry and of Experimental Endocrinology, University of Copenhagen, and Department of Insulin NOVO Ltd. Laboratories, Copenhagen, Denmark

Quantitative determination by means of the rat diaphragm or fat pad technique of insulin-like activity of human serum proteins indicates the insulin-like activity to be localized in the α- and in the γ-areas of preparative starch-block electrophoresis(1). The insulin-like activity can be localized by euglobulin precipitation in the precipitate; thus most probably a linkage exists between insulin and one or more serum proteins. Data given below which support this view can be obtained by means of immuno-electrophoresis. This method, when combined with autoradiography, can reveal protein binding of drugs, vitamins and hormones(2,3).

Material and methods. Immuno-electrophoresis was performed as described by Scheidegger(4). Autoradiography was made as described by Clausen & Munkner(2,3). Crystalline human insulin, labeled with J^{125} was produced by iodination in an acid medium(5). No significant amounts of radioactive impurities were present in the labeled insulin since: 1) when mixed with carrier pig insulin and subjected to starch-block electrophoresis the protein corresponded to the radioactivity pattern; 2) when mixed with carrier human insulin and subjected to recrystallizations the specific radioactivity remained constant.

The labeled insulin-J^{125} had after recrystallization a specific activity of about 0.6 mc/mg. One milliliter of normal human serum was mixed with about 10,000 micro units of insulin-J^{125}. The mixture was left for 4 days either at 4°C or at 22°C. Afterwards the serum was kept deep frozen until use. A similar experiment, performed with another batch with specific activity of about 8 mc/mg (but less purified through the preparation and hence containing considerable amounts of radioactive impurities), was carried out by adding 1000 μ units/ml serum.

Immuno-electrophoresis was performed in veronal buffer (pH = 8.6, ionic strength μ = 0.05). The immuno-electrophoreses were developed with a horse antiserum against normal pooled human serum from the Pasteur Institute, Paris, a goat antiserum against normal pooled human serum described earlier (6) and finally with specific antisera against α-2- and β-2-macroglobulin from Behring-