new epithelium.

The significance of glycogen deposition in squamous metaplasia of the uterus following vit. A deficiency and the biochemical enzyme systems involved is not known at the present time. There is evidence that the most important single factor which determines the rate of mitosis in tissue, such as mammalian epidermis, is the energy supply within the cells which depends upon the intracellular availability of glucose derivatives(5). If it can be assumed that because of increased mitotic activity the energy requirements of metaplastic epithelial cells is greater than for normal uterine epithelial cells, then the glycogen present in the metaplastic cells may be a source of readily utilizable energy.

The present results on glycogen deposition in epithelial metaplasia differs from the observation made in the human(6). In one instance a single squamous metaplastic cervical gland of one patient was reported to be free of glycogen. Since there appears to be a difference in glycogen deposition in squamous metaplasia, investigations are under way to determine whether the deposition of glycogen in stratified squamous metaplasia is peculiar to the changes following vit. A deficiency or whether the deposition of glycogen occurs in all epithelial metaplasia.

Summary. In the epithelial metaplasia of the rat uterus that occurred following vit. A deficiency, glycogen was observed in the stratified epithelium. An earlier report showed that the normal uterine epithelium of the rat uterus contains no glycogen.

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Received July 19, 1956. P.S.E.B.M., 1956, v93.

Effect of Methotrexate Therapy upon Choriocarcinoma and Chorioadenoma. (22757)

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Folic acid is known to be essential for the growth of the female genital tract and for normal embryonic development. Hertz *et al.* demonstrated the inhibition of estrogen-induced growth in the chick oviduct and monkey uterus in animals maintained on a folic acid deficient diet or treated with antagonists of either folic acid or adenine(1,2,3). Reduction of a previously high titre of gonadotropin occurred following a short course of methotrexate in a hypophysectomized woman with metastatic melanoma.*

The present report includes observations on the effect of a repeatedly administered and intensive regimen of methotrexate upon the urinary excretion of chorionic gonadotrophin and upon the clinical course in 2 patients with choriocarcinoma and one patient with chorioadenoma destruens.

Methods. The diagnosis was established by histological evidence. The initial extent of the disease was determined by physical examination and by radiological survey. Other observations included recording on each day of (a) fluid intake and output, (b) caloric intake, (c) the hemogram, and (d) the body weight. Each week x-rays were taken and measurement of palpable or visible lesions was recorded. Urinary gonadotropin determinations were carried out by a modification of the method of Klinefelter *et al.*(4). Gonadotropin values are expressed as "mouse uterine units" per 24 hours. The titres on

* Unpublished.

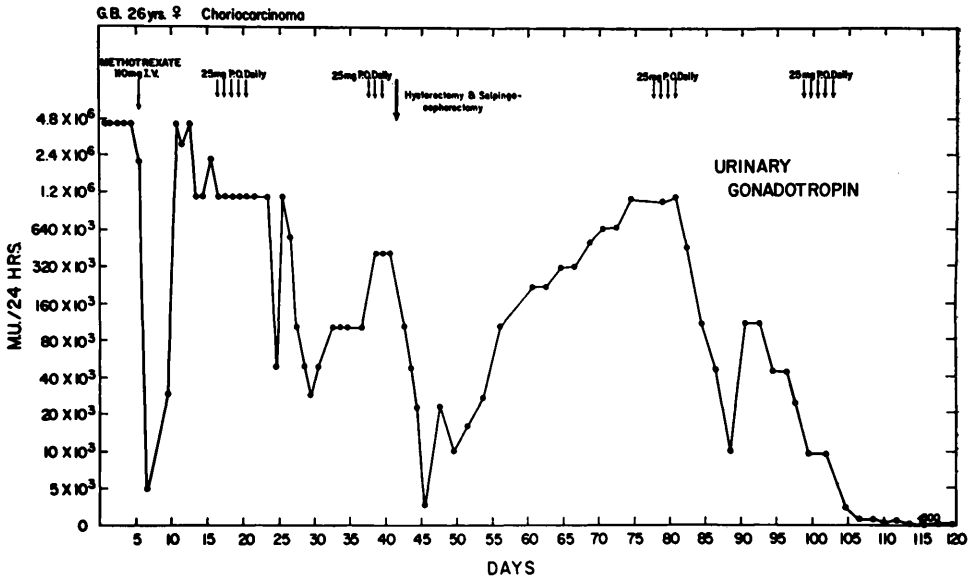

FIG. 1. Effect of intensive methotrexate therapy on urinary gonadotropin: Case 1.

graphs represent highest dilution yielding a positive reaction and twice this dilution was usually found to be negative. *Therapeutic Regimen*: The dosage regimen† of methotrexate is shown in the accompanying charts (Fig. 1, 2 and 3.) Two weeks after an initial single intravenous dose of 1 to 4.5 mg/kg body weight, there is given repeatedly at about 2 week intervals additional courses of 2.5 mg/kg body weight divided in 5 consecutive daily doses. The final course was given after the urinary gonadotropin titre had fallen to levels approaching normal with a view to a more sustained effect. Daily appraisal of the patient's hemogram, renal function, and clinical status with respect to stomatitis, glossitis, diarrhea, proctitis, and petechiae preceded the administration of the next dose. New courses were started only after clear evidence of complete hematological and gastrointestinal recovery from previous toxic effects.

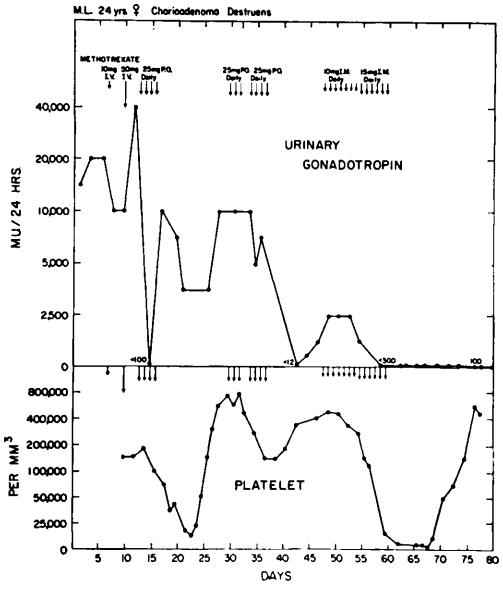

FIG. 2. Same as Fig. 1, and circulating platelet count: Case 3.

Results. By close adherence to the above mentioned precautions no undue toxicity was noted in these 3 patients. Experience with this same regimen (Fig. 3) emphasizes the possibility of renal impairment and this should be carefully checked by frequent BUN determinations and urinalyses. Moreover, preexisting renal impairment would constitute a definite contra-indication to the use of this regimen especially since any renal impairment would delay excretion and thereby enhance toxicity. Hepatic injury has not been observed.

† This regimen represents modification of procedure for intensive methotrexate administration devised by Dr. Paul T. Condit, Clinical Pharmacol. and Exp. Therap. Section, National Cancer Institute.

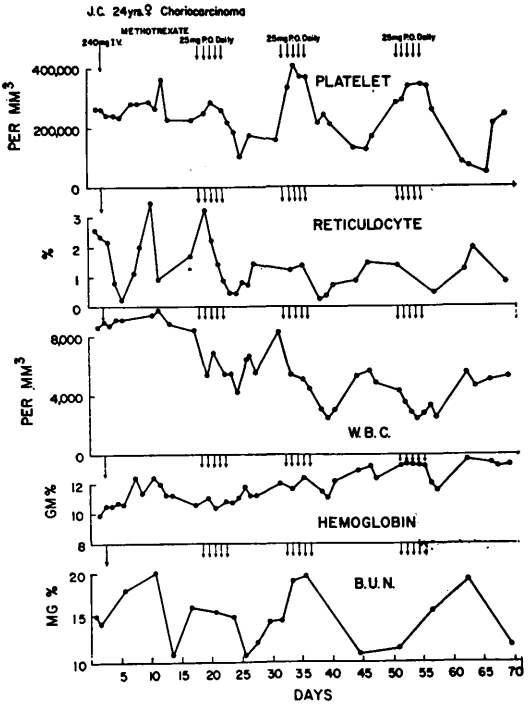

FIG. 3. Effect of intensive methotrexate therapy on the homopoietic elements and renal function: Case 2.

Fig. 2 and 3 illustrate transient impairment of hemopoiesis with each course of methotrexate. These effects began toward the close of each course and became most marked on the 4th to 10th day but full recovery was usually noted within 14 days.

Gastrointestinal difficulties such as anorexia, nausea, diarrhea, stomatitis, glossitis, ulceration of oral and rectal mucosa usually appeared and receded at about the same time as the bone marrow effects. These evidences of toxicity appeared to become progressively more apparent with each course of therapy.

Case 1. G.B., 26-year-old white housewife. She had a large pelvic mass and multiple bilateral pulmonary metastases. Diagnosis of choriocarcinoma had been established on tissue obtained by curettage. This case had been accepted for registration as choriocarcinoma in the Mathieu collection. On admission the patient had marked anorexia, protracted vomiting, and repeated bouts of uterine hemorrhage. There was evidence of marked weight loss and general debility. The urinary gonadotropin assay was positive at 4,000,000 mouse units/24 hours. The 5 courses of methotrexate given are indicated in Fig. 1. During the third course of methotrexate uterine bleeding became sufficiently profuse to indicate an emergency hysterectomy and salpingo-oophorectomy. Recovery was uneventful and further methotrexate therapy became indicated when the urinary gonadotropin titre had returned in a 6 weeks period from an immediate post-operative titre of 2,500 mouse units to 1,000,000 mouse units. After each course of methotrexate the gonadotropin titre was markedly suppressed (Fig. 1). With each successive course both the degree and duration of the gonadotropin reduction became greater. Following the 5th course the titre had receded to below 500 mouse units and has now remained at this reduced level for 5 weeks.

Correlated with this recession in urinary gonadotropin titre there was noted unequivocal x-ray evidence of regression of the pulmonary lesions (Fig. 4) and distinct amelioration of the patient's general clinical status.

Case 2. J.C., 24-year-old white housewife. She had a large, fixed, and irregular pelvic mass extending to the level of the umbilicus. She also had a globoid mass measuring 3 x 4 cm arising in the left labium major. Chest x-ray revealed diffuse bilateral pulmonary metastases. There had been extended vaginal hemorrhage and the patient was severely debilitated and critically ill. Several biopsy specimens showed choriocarcinoma with large masses of neoplastic cells presumably of trophoblastic origin. No villi were noted. The urinary gonadotropin titre was 200,000 mouse units/24 hours. Patient received 4 successive courses of methotrexate therapy. After the 4th course of therapy the gonadotropin titre had receded to below 200 mouse units and has remained at this level for the ensuing 6 weeks.

Accompanying this recession in gonadotropin titre the pelvic mass decreased to half its initial size and the labial mass was completely resolved. X-rays revealed striking clearing of the pulmonary lesions. The patient was completely rehabilitated and re-

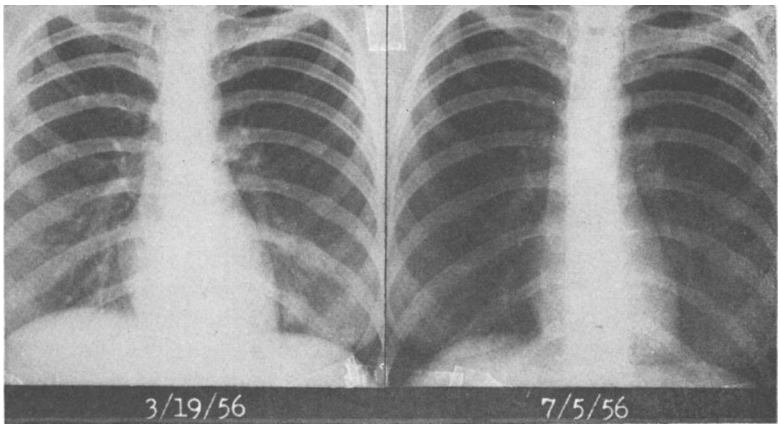

FIG. 4. Regression of pulmonary metastases. Case 1. Methotrexate therapy initiated 3/12/56.

mains under observation at this time on an ambulatory basis.

Case 3. M.L., 24-year-old housewife. Six months prior to admission she had undergone hysterectomy and bilateral salpingo-oophorectomy. The confirmed histological diagnosis was chorioadenoma destruens. On admission she was critically ill due to rapid progression of radiologically demonstrable pulmonary metastases. The urinary gonadotropin titre was 20,000 mouse units/24 hours.

Fig. 2 presents the therapeutic regimen and the associated changes in gonadotropin excretion in this case. It will be noted that the titre had dropped to below 300 mouse units and the patient experienced complete clinical recovery. During the past 8 months her gonadotropin titre has been found at monthly intervals to range between 100 and 200 mouse units. This titre is normal in our laboratory for an ovariectomized patient. Monthly x-rays (Fig. 5) show no evidence of metastatic disease since March this year.

Discussion. The correlation between clinical course and histopathological findings is notoriously confused in the case of trophoblastic tumors. Novak(5) and numerous others have described the pitfalls attendant upon clinical prognostication in this group of diseases on the basis of even the most expert histopathological analysis. Accordingly, we do not propose to draw any definitive therapeutic conclusions on the basis of the limited observations reported here. Nevertheless, the association of the as yet benign clinical course of these 3 patients with the methotrexate-induced recessions of urinary gonadotropin titre and regression of pulmonary metastases indicate the desirability of more extended study of the therapeutic potentialities of intensive treatment with antifolic and related compounds in this group of diseases.

Thiersch and Phillips(6) observed that the placental tissue in aminopterin-treated rats were not especially affected. This brings up the question whether methotrexate therapy employed here may have more general clinical application than in the type of cases just described. However, previous clinical experience with less intensive anti-folic regimens in several types of neoplasms other than acute leukaemia has not been encouraging(7). In any event, these observations emphasize the experimental advantage of an available index of tumor activity such as gonadotropin excretion in evaluation of therapeutic agents.

The intensive regimen of methotrexate therapy with courses interposed between successive periods of recovery from gastrointestinal and hematological toxicity is predicated upon the expected reversibility of such effects in normal tissues and possible failure of such reversibility in tumor tissue after repeated courses. The behavior of the urinary gonadotropin titres in these cases suggests that this expectation may have been realized. More prolonged follow-up in more cases will be required for further evaluation of such a rationale.

Caution was taken to ascertain whether the

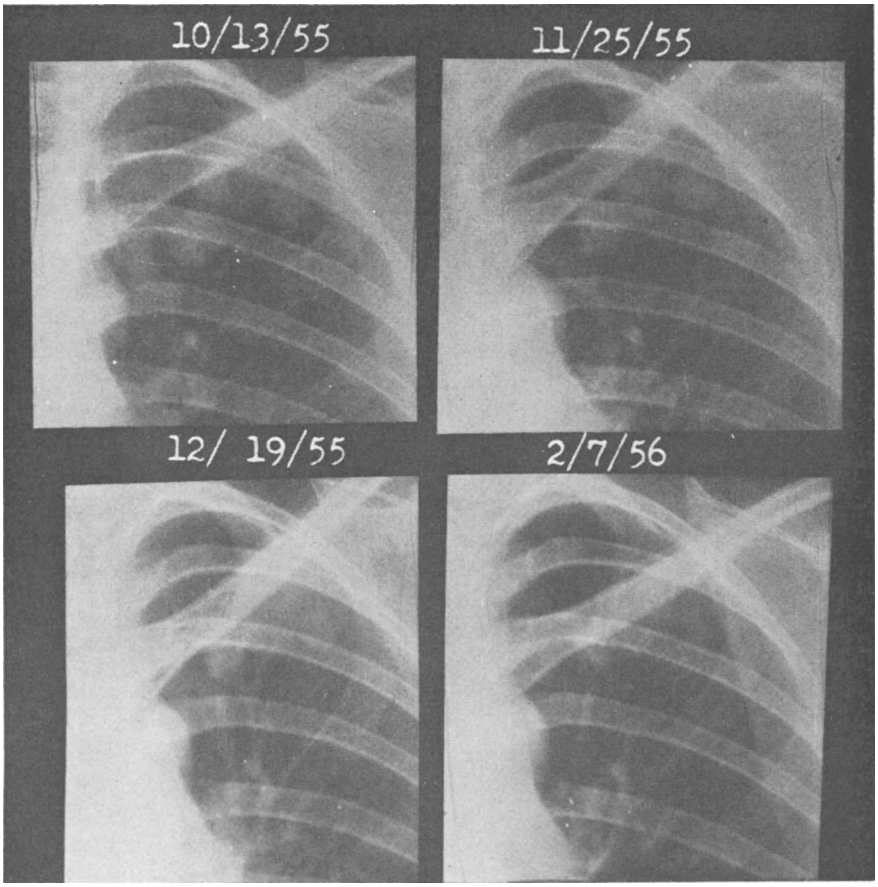

FIG. 5. Regression of pulmonary metastases. Case 3. Methotrexate therapy initiated 10/20/55.

apparent reduction in urinary gonadotropin may not have resulted from interference of excreted methotrexate and its metabolites with the biological assay. Addition of methotrexate to high titre urines did not alter the titre obtained probably because the dialysis of the urinary concentrate effectively removed substances of small molecular weight. Moreover, the persistence of the reduced titres for prolonged periods following cessation of therapy would render the possibility of such chemical interference unlikely.

Summary. 1) Two patients with choriocarcinoma and one with chorioadenoma destruens with proven progressive metastases were treated with methotrexate, a folic acid antagonist, according to intensive regimen of repeated courses of dosages producing substantial but reversible toxicity. Initially high urinary gonadotropin present in all 3 cases approached normal levels following this regimen and such low levels were sustained following cessation of treatment. 2) Unequivocal clinical improvement and radiological evidence of regression of pulmonary metastases was observed in all 3 cases. Nevertheless, extreme variations in pattern of clinical behavior of this type of tumor necessitate more observations before any definitive therapeutic implications may be drawn from these studies.

We are deeply grateful to Dr. Delbert M. Bergenstal, to Dr. Gordon Zubrod, and to Dr. Herbert A. Lubs for their valuable criticisms.

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Received July 26, 1956. P.S.E.B.M., 1956, v93.

Anti-Inflammatory Protection of Guinea Pigs by Egg Yolk: Effect on Adrenals and Serum Constituents. (22758)

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Coburn *et al.*(3) have shown that dietary egg yolk reduces the intensity of the inflammatory response induced by passive anaphylactic arthritis in young guinea pigs. Their observations indicated that the active material of egg yolk resides in the alcohol soluble (crude phosphatide) fraction and is absent from both the acetone solubles (neutral fats and cholesterol) and the yolk protein. We have confirmed the above findings(19) using the anti-inflammatory bio-assay method of French *et al.*(8). The adrenal hormone, cortisone, and its related steroids have been used in treatment of inflammatory diseases(15,18). Their protection of laboratory animals against inflammation induced by BCG(21), tuberculin(2), turpentine(16), and Pneumococci(22) has also been demonstrated. French *et al.* (8) showed that cortisone treatment also reduced swelling in joints of guinea pigs which had been subjected to a reverse Arthus reaction.

The present investigation was undertaken (a) to compare the anti-inflammatory protection by cortisone to that afforded by dietary egg yolk, (b) to study the effect of yolk alcohol solubles on adrenals of the guinea pig, and (c) the effect of egg yolk on blood constituents of guinea pigs.

Methods. 1. *Fractionation of egg yolk powder.*† Acetone solubles, alcohol solubles and the protein fraction of egg yolk were obtained by procedures of Coburn *et al.*(3). 2. *Assaying procedure.* Weanling guinea pigs weighing about 160 g (unless otherwise indicated) were used as test animals in groups of 6 or more. Each animal was weighed just prior to, and every 3 days during the experiment, and daily food intake was recorded. Egg yolk and yolk fractions were individually fed to guinea pigs as supplements to a basal control diet for a period of 21 to 26 days. The basal diet contained 50% Rockland guinea pig diet and 50% dixie rabbit pellets (22% protein) to which was added 0.1% ascorbic acid. At end of the feeding period, the guinea pigs usually reached the desired weight of approximately 300 g and then used for bio-assay(8). For each assay group, a control group of 6 or more guinea pigs was fed and challenged at the same time to minimize environmental influences on inflammation.

Results. Exp. 1 was a comparison of the anti-inflammatory activity of egg yolk and yolk alcohol solubles to that of cortisone. Cortisone acetate was injected intraperitoneally into 300 g guinea pigs (after 3-4 days on basal diet) 1 hr prior to assay. For each assay a second group of guinea pigs, pretreated with saline, was used as control. Feeding dried egg yolk at 19.5%, or yolk alcohol solubles at 2-8% of diet, resulted in 28-40% inhibition of swelling (Table I) similar to that obtained by administering cortisone acetate at the level of 25 mg/kg body weight(8). When alcohol solubles were fed at less than 2% of diet, no protection was observed.

Exp. 2 was a study of the effect of dietary yolk alcohol solubles on adrenals of the guinea

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