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Urinary Excretion of Estrogens and 17-Ketosteroids in Young, Adult Males With Infectious Hepatitis.

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Male subjects with chronic diseases of the liver frequently display symptoms which are best explained by assuming an increased concentration of circulating, biologically active estrogenic compounds. Testicular atrophy,[1] gynecomastia, decrease in total body hair, development of spider telangectasia, feminine distribution of pubic hair, and loss of libido,[2,3,4] include some of the phenomena associated with cirrhosis of the liver in the male. In some of these cases a high output of urinary estrogen has been recorded, along with a fall in the total excretion of urinary androgens.

Numerous experiments with animals have shown that the liver inactivates, or otherwise disposes of large quantities of exogenous and endogenous estrogens.[5-11] If estrogenic compounds are injected into the portal circulation, their systemic effects are negligible compared to the effect of a similar quantity of hormone injected into extra-portal areas.[12,13,14] At least 2 major roles have been assigned to the liver in the metabolism of estrone; one, that it is rapidly effective in converting estrone to estradiol, and secondly, that it acts to convert all estrogenic materials to compounds which are no longer phenolic in character.[9] These data provide a theoretical basis for explanation of the marked degree of feminization occasionally seen in males with advanced cirrhosis of the liver[15,16] and suggest that estrogen metabolism may be significantly impaired in chronic hepatic insufficiency.

In the present study an attempt has been made to learn whether defective estrogen metabolism likewise occurs in acute infectious hepatitis. At the same time studies on the urinary excretion of the total 17-ketosteroids have been carried out in order to learn if the metabolism of androgens may also be affected in acute liver insufficiency. The results of serial studies of the urinary excretion of estrogens and 17-ketosteroids in 11 males with infectious hepatitis are recorded in the accompanying graph (Fig. 1). All patients selected for this study were young adult males, previously in good health, and with no demonstrable diseases other than hepatitis. The number of days following the appearance of the first symptoms are recorded on the abscissa. The concentration of free urinary estrogen and total 17-ketosteroids, in micrograms and milligrams per 24 hours, respec-

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1 Morrione, T. G., *Arch. Path.*, 1944, **37**, 39.

2 Edmondson, H. A., Glass, S. J., and Soll, S. N., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 97; Glass, S. J., Edmondson, H. A., and Soll, S. N., *Endocrinology*, 1940, **27**, 749.

3 Glass, S. J., Edmondson, H. A., and Soll, S. N., *J. Clin. Endocrinology*, 1944, **4**, 54.

4 Sheldon, J. H., *Lancet*, 1934, **2**, 1031.

5 Zondek, B., *Skandinav. Arch. f. physiol. Chem.*, 1934, **70**, 133.

6 Heller, C. G., and Heller, E. J., *Endocrinology*, 1943, **32**, 64.

7 Talbot, N. B., *Endocrinology*, 1939, **25**, 601.

8 Biskind, M. S., and Biskind, G. R., *Endocrinology*, 1942, **31**, 109.

9 Schiller, J., and Pincus, G., *Endocrinology*, 1944, **34**, 203.

10 Longwell, B. B., and McKee, F. S., *J. Biol. Chem.*, 1942, **142**, 757.

11 Israel, S. L., Meranze, D. R., and Johnston, C. G., *Am. J. Med. Sci.*, 1937, **194**, 835.

12 Golden, J. B., and Sevringhaus, E. L., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 361.

13 Biskind, G. R., and Meyer, M. A., PROC. SOC. EXP. BIOL. AND MED., 1943, **53**, 91.

14 Segaloff, A., *Endocrinology*, 1943, **33**, 209.

15 Kyrle, J., *Verhandl. d. deutsch. path. Gesellsch.*, 1909, **13**, 391.

16 Weichselbaum, A., *Verhandl. d. deutsch. path. Gesellsch.*, 1910, **14**, 234.

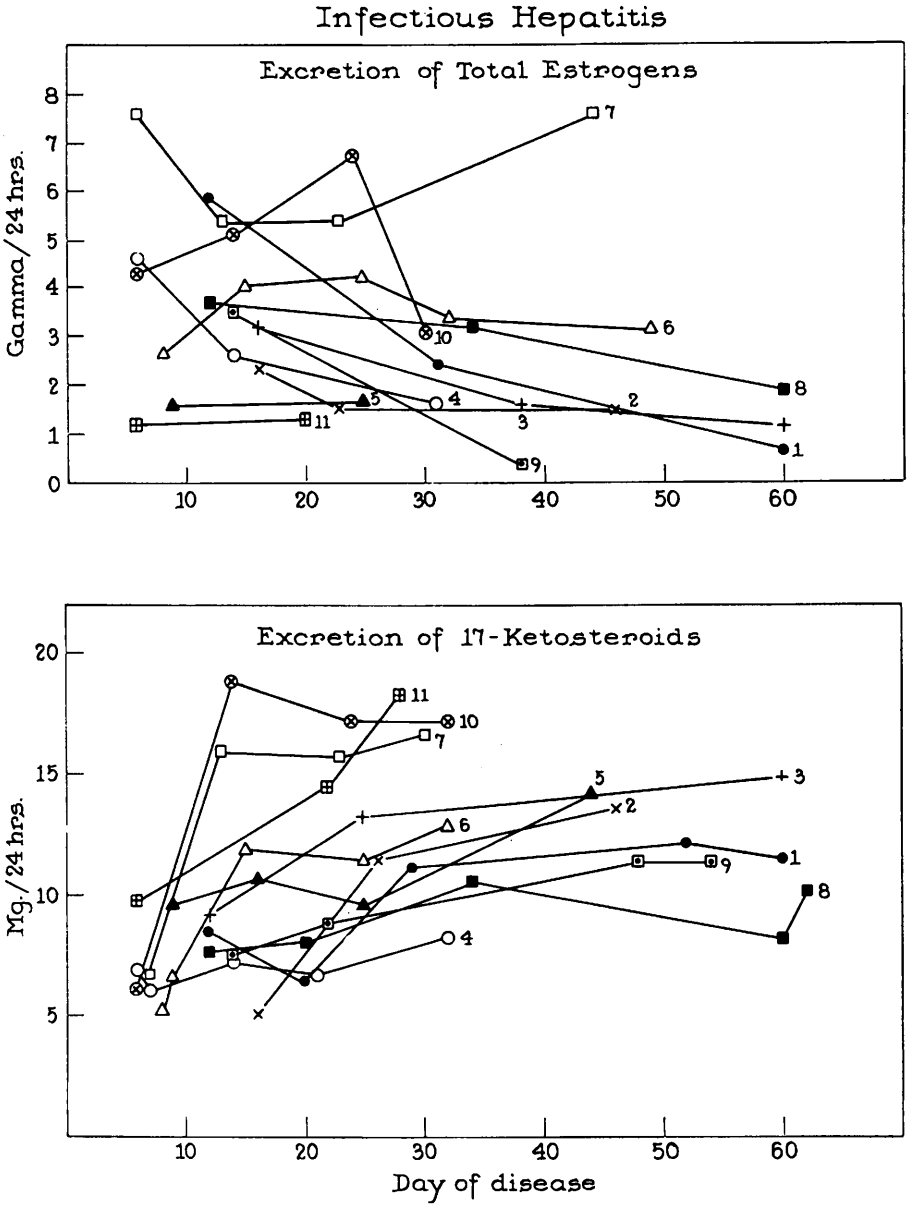

FIG. 1.

Graph showing values for the urinary excretion of estrogens and 17-ketosteroids in young, adult males with infectious hepatitis.

tively, has been plotted on the ordinate. The estrogen assays were carried out by a modification of the Allen-Doisy technic,[17,18] in which 0.1 cc of a urine extract prepared by hydrolysis, extraction with carbon tetrachloride, and concentration of the phenolic compounds in the standard manner, was injected 4 times in 48 hours into spayed mice, and vaginal smears studied on the third day. An average of 15 animals were used for each urine extract. The method for the 17-ketosteroids was essentially

[17] Thayer, S. A., Doisy, E. A., Jr., and Doisy, E. A., *Yale J. Biol. and Med.*, 1944, **17**, 19.

[18] Allen, E., and Doisy, E. A., *J. Am. Med. Assn.*, 1923, **81**, 819.

that of Talbot,[19] with modifications suggested by Dr. Ephraim Shorr.[20] In general the methods proved to be reliable, and the results reproducible.

The range of excretion of estrogens in normal males is low, and quite varied. Moreover, the values depend to a great extent upon the technic employed for the preparation of the urinary extract and upon the method used for assay. It was decided, therefore, to follow the estrogen excretion of each patient at frequent intervals throughout the course of the disease, and to use as a control base line for each case the quantity of estrogenic substances excreted following recovery from hepatitis.

With the convalescent level as a control, it may be seen that in nearly every case there was a significant rise in estrogen excretion during the acute phase of hepatitis, and that a diminishing elevation in estrogen excretion was maintained until relatively late in convalescence. In Subject 7, values for the urinary excretion of estrogens were high within the first 5 days following the onset of the disease process. By the 12th day an abrupt fall had occurred, associated with marked clinical improvement. No further drop in estrogen excretion was observed. However, on the 45th day after onset of the disease, a significant rise in the excretion of urinary estrogens had again occurred, coincident with a return of clinical signs of hepatitis. Following clinical recovery of the patient, normal values were recorded for the urinary excretion of estrogens of 2.9 μg per 24 hours. Delayed clinical recovery in another patient with hepatitis, Case No. 6, was likewise reflected in a persistent moderate elevation of urinary estrogens. Further studies, attempting to relate persistent elevation in urinary estrogen excretion to delay in convalescence and chronicity in infectious hepatitis are under way, and the results will be published subsequently.

In most cases there appeared to be a significant correlation between the severity of the attack of acute hepatic insufficiency, as judged from the results of clinical and laboratory studies, and the estrogenic activity of the urine. For example, Case No. 5 and No. 11, in whom normal values for the urinary excretion of estrogens were recorded during the course of the disease, presented exceptionally mild symptoms of infectious hepatitis, requiring only 20 and 28 days, respectively, for complete clinical recovery.

The normal range of the urinary level of 17-ketosteroids in males, like that for the estrogens, is variable, with most reports placing values at 8-28 mg per 24-hour period of urinary excretion. Again, because of the wide variation in the normal level of androgen excretion, it was considered that the convalescent level of 17-ketosteroids provided the best possible normal base line for each patient, and the most effective standard for a comparison of the degree of aberration in 17-ketosteroid excretion occurring in each case during the acute and convalescent states of infectious hepatitis.

The urinary excretion of 17-ketosteroids in the majority of cases was low in the early stages of hepatitis, and rose to a normal level during the period from the 20th to the 50th day of the disease, where it remained approximately constant. In some cases the level of excretion was more than twice the value obtained during the acute stages of the disease. In 2 cases in whom there occurred recrudescence of hepatitis with a return of clinical symptoms, the 17-ketosteroid levels of the urine fell again to values comparable to those observed during the first acute attack of the disease. In both cases clinical recovery was followed by an elevation of the urinary 17-ketosteroids to normal values.

At first glance there appears to have been a reciprocal relationship between the levels of excretion of estrogens and 17-ketosteroids in the urine of patients with acute hepatic insufficiency. Closer analysis, however, reveals that in all cases, a rise in 17-ketosteroids, often to a normal level, occurred considerably in advance of a significant decrease in the level of urinary estrogens. The excretion of 17-ketosteroids is temporarily depressed in a variety of conditions, including fasting, protein

[19] Talbot, N. B., Berman, R. A., MacLachlan, E. A., and Wolfe, J. K., *J. Clin. Endocrinology*, 1941, **1**, 668.

[20] Shorr, E. P., personal communication.

depletion, and general debility. It is quite possible, therefore, that the depression of the excretion of 17-ketosteroids observed in acute hepatic insufficiency may have resulted from the secondary effects of the disease, rather than to a rise in the level of active circulating estrogens. That reciprocal depression of androgen formation may occur eventually, as a result of the failure of the liver to accomplish the inactivation of estrogenic compounds, is a definite possibility, and may conceivably account for the persistent diminution in the excretion of 17-ketosteroids which has been observed in chronic hepatic insufficiency.

Summary. The levels of excretion of estrogens and 17-ketosteroids in the urine have been determined in 11 young adult males with acute infectious hepatitis, at frequent intervals, from onset of the disease to convalescence. In 9 moderately severe cases there was a significant elevation in the level of excretion of biologically active estrogens in the urine which persisted at a slowly diminishing elevation until convalescence was considerably advanced. There was no significant increase in the concentration of the urinary estrogens in 2 cases of hepatitis which presented only mild clinical symptoms.

Marked to moderate depression occurred in the level of excretion of urinary 17-ketosteroids early in the course of acute hepatitis, with a return to normal values considerably in advance of the period of diminished estrogen excretion and of clinical recovery.

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Adequacy of the Known Synthetic Vitamins for Normal Feathering and Pigmentation in Chicks.

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The existence of a factor, or factors, in liver or yeast other than pantothenic acid, *p*-aminobenzoic acid, biotin, or inositol necessary for normal pigmentation was indicated from earlier work in this laboratory with rats[1,2] and dogs.[3] McGinnis, Norris, and Heuser[4] likewise indicated the presence of an unidentified factor other than pantothenic acid in brewers' yeast which was necessary for normal feathering and pigmentation in growing Rhode Island Red chicks. Cure or prevention of the achromotrichia developed in rats fed sulfaguanidine or succinylsulfathiazole by administration of folic acid concentrates was reported by Martin[5] and by Wright and Welch.[6] Similar results have been obtained in this laboratory using liver concentrates. It appeared, therefore, that the active factor might be identified with the *L. casei* factor[7] and vitamin B_c.[8]

Attempts have been made to develop techniques capable of assay for the unknown pigmentation factor. Chicks and dogs on highly purified diet and rats on purified diet with added succinylsulfathiazole have been used. Most consistent results have been obtained with growing chicks. In studying the role of the *L. casei* factor in hair and feather pigmentation, concentrates of the vitamin prepared by one of us (F.C.M.) and finally the

[1] Frost, D. V., Moore, R., and Dann, F. P., PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 507.

[2] Dann, F. P., Moore, R., and Frost, D. V., *Fed. Proc. Soc. Exp. Biol. and Med.*, 1942, **1**, 107.

[3] Frost, D. V., and Dann, F. P., *J. Nutrition*, 1944, **27**, 355.

[4] McGinnis, J., Norris, L. C., and Heuser, G. F., *J. Biol. Chem.*, 1942, **145**, 341.

[5] Martin, G., PROC. SOC. EXP. BIOL. AND MED., 1942, **51**, 353.

[6] Wright, L. D., and Welch, A. D., *J. Nutrition*, 1944, **27**, 55.

[7] Pfiffner, J. J., *et al., Science*, 1943, **97**, 404.

[8] Angier, R. B., *et al., Science*, 1945, **102**, 227.