completely prevented the migration of mammalian macrophages(1).

The action of endotoxin on large wandering cells in cultures of lung was similar to that in cultures of spleen in the present study but attempts to culture chicken lung were usually unsuccessful due to bacterial contamination. Few migrating cells appeared in cultures of liver, and none were observed in cultures of testis.

Summary. Different concentrations of endotoxin were added to cultures of adult chicken's spleen growing in a coagulated plasma medium. Endotoxin in concentrations of 0.1 and 1.0 μg/ml did not alter the early migration of leucocytes and macrophages, but stimulated the extent of macrophage migration after the second day of incubation. Concentrations of endotoxin between 5 and 50 μg/ml caused an initial decrease in macrophage migration followed by a definite increase in a majority of experiments. Morphologic changes due to endotoxin were not observed. The low toxicity of endotoxin for avian macrophages was different from the severe toxic action on mammalian macrophages previously observed under similar cultural conditions.

1. Heilman, D. H., Bernton, H. W., *Am. Rev. Resp. Dis.*, 1961, v84, 862.

2. Heilman, D. H., *Fed. Proc.*, 1963, v22, 628.

3. ———, *Bacterial Endotoxins,* M. Landy and W. Braun, Eds., Rutgers Univ. Press, New Brunswick, N. J., 1964, p610.

4. Ball, R. A., Sautter, J. H., Burger, R. E., Pomeroy, B. S., PROC. SOC. EXP. BIOL. AND MED., 1962, v110, 753.

5. Jordan, M. M., Hinshaw, J. B., *ibid.*, 1964, v115, 455.

6. Meier, R., Schär, B., *Experientia*, 1953, v9, 93.

7. ———, *ibid.*, 1954, v10, 376.

8. ———, *Arch. Exp. Path. u Pharmakol.*, 1958, v234, 102.

9. Schmidt, G., Eichenberger, E., Westphal, O., *Experientia*, 1958, v14, 289.

10. Meier, R., Schär, B., Braun, W., *Helv. Physiol. Pharmacol. Acta*, 1960, v18, 266.

11. Schär, B., Meier, R., *Experientia*, 1961, v17, 27.

12. Barg, G. Z., *Z. Immunitaetsforsch.*, 1932, v74, 372.

13. Lasfargues, E. Y. L., Whorton, D. R. A., DiFine, J. C., *Cancer Res.*, 1951, v11, 425.

14. Gabliks, J., Solotorovsky, M., *J. Immunol.*, 1962, v88, 505.

15. Heilman, D. H., Feldman, W. H., Mann, F. C., *Am. Rev. Tuberc.*, 1944, v50, 344.

16. Heilman, D. H., *Int. Arch. Allerg.*, 1964, in press.

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Dissociation of Hypophyseal Content and Urinary Excretion of Gonadotropin in Cirrhosis.* (29438)

AGNES B. RUSSFIELD, EDWIN R. FISHER AND SHELDON C. SOMMERS

Children's Cancer Research Foundation, Boston, Mass., Departments of Pathology, Univ. of Pittsburgh, Pa., and Columbia University College of Physicians and Surgeons, New York

By a collaborative investigation of pituitary bioassays and urinary gonadotropins in cirrhotic men, an attempt has been made to resolve the common paradox of estrogenization, testicular atrophy and pituitary basophil hyperplasia that is observed at autopsy (1-4). Increased percentages of anterior hypophyseal basophil cells might have been anticipated to indicate ample gonadotropic hormonal stimulation of the testes and to be associated with elevated levels of urinary gonadotropin. However, the cirrhotics actually have shown low urinary gonadotropins(2), and evidence of pituitary storage rather than secretion of gonadotropins.

Materials and methods. Pituitary glands collected at autopsy from 13 men with advanced hepatic cirrhosis were fixed in ice cold acetone, dried and stored at —20°C. Aliquots of powdered pituitary equivalent to 10 mg of fresh tissue were suspended in physiological saline solution, and these were in-

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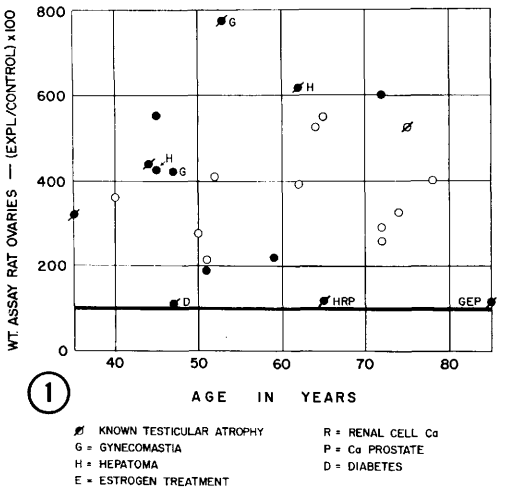
jected daily for 4 days into each of 3 immature hypophysectomized rats obtained from Charles River Farms. On the fifth day the rats were killed, the ovaries and uteri were weighed, sectioned and examined histologically for evidences of hormonal stimulation. Saline-injected hypophysectomized rats of similar age were likewise examined. Control human material was provided by 12 pituitary glands from men of comparable ages who died of coronary thrombosis and had no evidence of liver disease at autopsy.



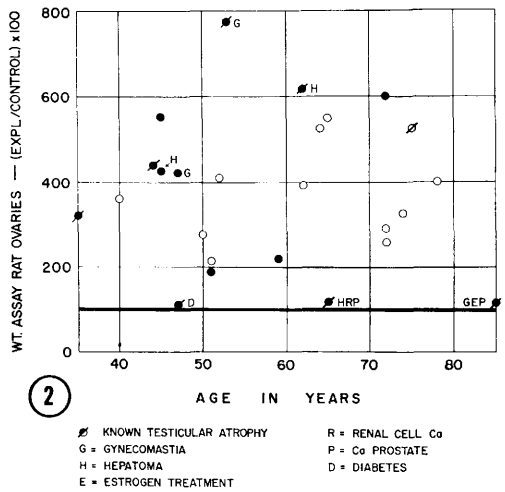


FIG. 1. Gonadotropic hormone storage in hypophyses of men dying with cirrhosis (●) and coronary artery disease (○).

FIG. 2. Gonadotropic hormone storage in hypophyses of men dying with cirrhosis (●) and coronary artery disease (○).

TABLE I. Urinary Gonadotropin Determinations in Cirrhotic Men.

Age of patient	Testicular atrophy	Gyneco-mastia	Gonadotropin excretion/24 hr, Mouse units 5	10	20	50	100
41	+	+	+	?	—	—	—
43	+	0	+	+	+	—	—
49	+	0	—	—	—	—	—
67	+	0	—	—	—	—	—
73	?	0	+	—	—	—	—
26	0	0	—	—	—	—	—
39	0	0	+	±	—	—	—
44	0	0	—	—	—	—	—
69	0	0	—	—	—	—	—

Specimens of 24-hour urine were collected from 9 men hospitalized for advanced cirrhosis, none included in the autopsied cases. Both groups had predominantly nutritional cirrhosis. Gonadotropins were absorbed on Kaolin, eluted and assayed by the method of Klinefelter and co-workers, using the immature mouse uterus(5).

Results. As shown in Fig. 1, the cirrhotic and control cases usually had comparable amounts of stored hypophyseal gonadotropic hormone, judged by the weight gain of rat ovaries and confirmed by the ovarian histologic changes. These qualitative assays usually showed from 200 to over 700% comparative increases in ovarian weight, as judged by the controls. The highest value occurred in a man aged 75 with a previous unilateral orchiectomy performed during herniorrhaphy.

Three cirrhotics had no pituitary gonadotropin storage demonstrated. They included a 47-year-old diabetic who died in acidosis from hematemesis; a 65-year-old man who died in shock from hemorrhage associated with renal cell carcinoma, and with an incidental hepatoma and prostatic carcinoma; and a man 86 who had received prolonged Stilbestrol therapy for prostatic carcinoma.

Urinary gonadotropin determinations (Table I) were negative in 5 of the 9 cirrhotic men. Two of these had clinical testicular atrophy. Three additional cirrhotics excreted 5 mouse units of gonadotropin per day, and one had both clinical testicular atrophy and mild gynecomastia. The last cirrhotic, who excreted 20 mouse units per day, had testicu-

lar atrophy but no gynecomastia.

No consistent pattern of gonadotropic or other pituitary hormone storage was found in the cirrhotic men that could be correlated with the presence of gynecomastia, hepatoma or other malignant tumors.

Discussion. Within the acknowledged limitations of the variable human material available, the qualitative type of pituitary bioassay employed, and the complicated endocrine changes that may accompany cirrhosis, the observation of ample stored pituitary gonadotropin and low or absent urinary gonadotropin excretion is enlightening. Usually urinary gonadotropin excretion parallels the hypophyseal storage of gonadotropin, but in human cirrhosis there is a discrepancy. Also, in experimental starvation a dissociation of the hypophyseal gonadotropin content and circulating hormone has been reported(6).

The low pituitary contents of gonadotropin in 2 of the cirrhotics who died of hemorrhage and shock are unexplained, unless unusual stress had been associated with a rapid release of stored hormone. In the other man with low pituitary gonadotropin, Stilbestrol therapy might be implicated since several other patients treated with large doses of estrogens have had very low levels of pituitary gonadotropin storage(7). Malnourished humans have also showed both low levels of pituitary gonadotropin storage and urinary excretion(8,9).

Without excluding the possibility that in cirrhosis pituitary gonadotropins might be released and then inactivated before their urinary excretion, an explanation of these findings is proposed. In cirrhosis the moderately increased levels of circulating estrogen in men appear to block the secretion of pituitary gonadotropin, with a consequent reduction of the urinary excretion levels. In some other conditions, such as women with polycystic ovaries, the same apparent dissociation may occur between pituitary basophil hyperplasia and low or normal urinary gonadotropin levels(10,11).

Summary. Bioassays of gonadotropin from 13 cirrhotic men showed ample hypophyseal gonadotropic hormone in 10. Urinary gonadotropin excretion was not detected in 5 of 9 cirrhotics, and had low normal values in the other 4. Pituitary gonadotropin release may be inhibited in some cases of cirrhosis, possibly by estrogen.

1. Rather, L. J., *Arch. Int. Med.*, 1947, v80, 397.
2. Lloyd, C. W., Williams, R. H., *Am. J. Med.*, 1948, v4, 315.
3. Bennett, H. S., Bagenstoss, A. H., Butt, H. R., *Am. J. Clin. Path.*, 1950, v20, 814.
4. Barr, R. W., Sommers, S. C., *J. Clin. Endocrinol.*, 1957, v17, 1017.
5. Klinefelter, H. F., Jr., Albright, F., Griswold, G. C., *ibid.*, 1943, v3, 529.
6. Maddock, W. O., Heller, C. G., PROC. SOC. EXP. BIOL. AND MED., 1947, v66, 595.
7. Russfield, A. B., *Cancer*, 1960, v3, 790.
8. Russfield, A. B., Sommers, S. C., *Arch. Path.*, 1963, v75, 564.
9. Zubiran, S., Gomez-Mont, F., *Vitamins and Hormones*, 1953, v11, 97.
10. Sommers, S. C., Wadman, P. J., *Am. J. Obst. and Gyn.*, 1956, v72, 160.
11. Keettel, W. C., Bradbury, J. T. B., Stoddard, F. J., *ibid.*, 1957, v73, 954.

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Cross Reactions with Tuberculins on Guinea Pigs with Single and Double Experimental Infections with Mycobacteria.* (29439)

WILLIAM W. JOHNSTON AND DAVID T. SMITH
Departments of Pathology and Microbiology, Duke University Medical Center, Durham, N. C.

For many years, tuberculosis-like infections in man produced by mycobacteria other than *Mycobacterium tuberculosis* have been reported in the literature(1-3). In most cases these reports, consisted of descriptions of single cases or very small groups of cases, with

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