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Inhibitory Effect of Prolactin on the Development of Fatty Liver Induced by ACTH in the Rat[1] (38784)

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Among the various factors causing fatty infiltration of the liver hormonal effects are of particular interest. There is numerous evidence of the role of pituitary hormones, primarily of ACTH and GH, in the mobilization of depot fat (1–6). The effects of ACTH and GH upon the development of fatty liver include the enhancement of fat mobilization followed by the fatty infiltration of liver. Adrenalectomy has been shown to prevent fatty infiltration of the liver induced by various procedures (7–9).

The present study was undertaken to cast more light on the action and interaction of ACTH, GH and prolactin in the development of fatty liver. In our work special consideration was given to the action of prolactin upon lipid accumulation in the rat liver, since this hormone had been shown to influence lipid metabolism in the rat ovary (10), pigeon liver (11) and pigeon crop epithelium (12).

Material and Methods. Female rats of the Fischer strain aged 90 days were used. The rats were injected with commercial ACTH of prolonged action (Cortrophine-Z, Organon), GH (NIH-GH-B15 bovine, 0.9 IU/mg) and prolactin (NIH-P-S-9 ovine, 30 IU/mg) obtained from the Endocrine Study Section of NIH. ACTH was injected in daily doses of 4 and 12 IU (A_4, A_{12}), prolactin and GH in daily doses of 5 and 10 mg. The hormones were administered sc twice a day, over a period of 10 days.

Total lipids were determined by the gravimetric method after extraction in an ether-ethanol (1:3) mixture. Phospholipids were analyzed in the same ether-ethanol extract using the method of Outhouse *et al.* (13).

Results. Growth hormone or prolactin administered to intact adult female rats in daily doses of 5 and 10 mg alone or in combination elicited changes in liver total lipids content ranging from −5% (P_5) to +25% (GH_{10}) (Fig. 1). These effects are inappreciable in comparison to the effects of ACTH alone, 4 IU of which increased total lipids content by 101% (508 ± 27.7 vs 252 ± 14.2) and 12 IU by 131% (581 ± 40.7 vs 252 ± 14.2). Combinations of 5 mg GH and 4 or 12 IU ACTH were more effective than ACTH alone, causing total lipids content to increase by an additional 28% (651 ± 19.5 vs 508 ± 27.7) and 25% (726 ± 79.0 vs 581 ± 40.7), respectively. The smaller dose of prolactin combined with 4 IU ACTH produced a 28% decrease (415 ± 13.1 vs 508 ± 27.7) and combined with 12 IU ACTH a 31% decrease (459 ± 20.9 vs 581 ± 40.7) in total lipids content in comparison to ACTH alone. Simultaneous injections of 5 mg GH, 5 mg prolactin and 4 or 12 IU ACTH changed total lipids content by −3% and −10%, respectively, compared to the effects of ACTH alone.

Hormonal treatment elicited the same pattern of changes in liver total lipids concentration as in the liver total lipids content (Table I).

Injections of ACTH, GH or prolactin resulted in an increase in the liver phospholipid content. Combinations of ACTH and GH produced a greater increase than ACTH alone, while simultaneous injections of ACTH and prolactin had the same effect as ACTH alone. Administration of a combination of all three hormones caused similar effects as the combination of ACTH and GH (Table I).

The effect of the higher dose of ACTH upon adrenal gland weight was significantly reduced by simultaneous application of 5 mg GH (146 vs 181) or a combination of 5 mg GH and 5 mg prolactin (128 vs 181).

Discussion. Administration of ACTH or

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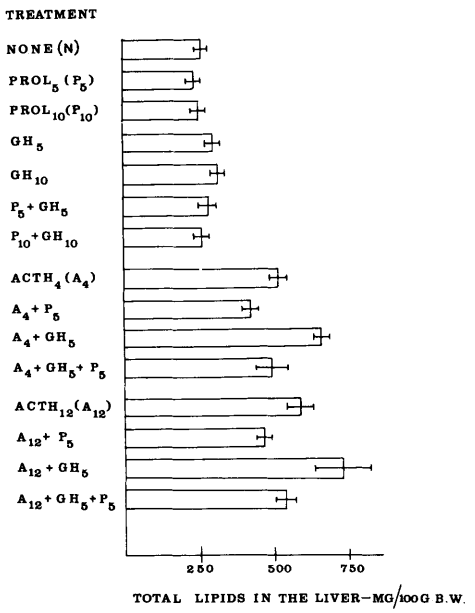

FIG. 1. Effects of ACTH, GH and prolactin on liver total lipids content in normal intact female rats. The hormones were injected sc twice a day for 10 days. Daily doses are indicated by numbers. Values are mean ± SE. Five rats per group were used, except for group N (12 observations) and GH_5 (10 observations). $A_4 + P_5 < A_4$, $P < 0.02$; $A_{12} + P_5 < A_{12}$, $P < 0.05$; $A_4 + GH_5 > A_4$, $P < 0.02$; $A_{12} + GH_5 + P_5 < A_{12} + GH_5$, $P < 0.05$; $A_4 + GH_5 + P_5 < A_4 + GH_5$, $P < 0.02$.

of combinations of ACTH and GH to intact female rats elicited significantly greater increase in liver total lipids content and total lipids concentration than combinations of ACTH, or ACTH and GH, with prolactin (Fig. 1, Table I). These results seem to suggest that prolactin partially inhibits the effect of ACTH promoting the development of fatty liver. It has been demonstrated that prolactin influences lipid metabolism in the rat ovary (10), pigeon liver (11) and pigeon crop epithelium (12). While in these studies prolactin was shown to influence lipid metabolism by stimulating fatty acid synthesis and lipid accumulation in the above mentioned tissues, our results obtained on rat liver indicate that prolactin has no effect on liver total lipids content when administered alone, but only in combination with ACTH, when it reduces the lipolytic effect of ACTH.

Unlike prolactin, which partially inhibited ACTH in its effect promoting the development of fatty liver, GH combined with ACTH potentiated the lipolytic effect of ACTH. In 1952 Levin and Farber (14) demonstrated that exogenous treatment of mice with combinations of cortisone and GH caused a more significant increase in mouse liver fat than administration of cortisone alone. These authors were of the opinion that GH might be a "triggering" substance which, in addition to corticoids, induces the mobilization of fat in the body and promotes thereby its deposition in the liver. They could not support their hypothesis on this possible role of GH with conclusive evidence, because the GH preparations available at that time were not chemically pure. Our data, obtained with more pure GH preparations, provide evidence that GH is capable of potentiating the effect of ACTH in the mobilization of fat. In our experiments GH injected alone produced slight accumulation of lipids in the liver. This finding is in agreement with the data reported by Greenbaum and McLean (15), but not with the hypothesis that GH alone is only capable of increasing the capacity of the adipose tissue to carry out lipolysis in response to lipogenic agents rather than initiating a lipolytic process (16).

The partial inhibitory effect of prolactin upon ACTH in the development of fatty liver and potentiation of the effect of ACTH by GH are very interesting phenomena in the light of the findings that these two hormones may produce similar effects in human (17) and rat (18) lipid metabolism. Our results indicate that prolactin and GH may have opposite effects upon ACTH in the process of mobilization of fat for deposition in the liver of the rat.

Summary. The action and interaction of ACTH and prolactin in the development of fatty liver were investigated in intact rats treated with exogenous hormones. Administration of ACTH or of combinations of ACTH and GH to intact female rats was found to elicit significantly greater increase in liver total lipids content and concentration than administration of combinations of ACTH, or ACTH and GH, with

TABLE I. EFFECTS OF HORMONES ON LIVER WEIGHT, TOTAL LIPIDS CONCENTRATION AND PHOSPHOLIPIDS CONTENT IN THE LIVER AND ON ADRENAL GLAND WEIGHT OF INTACT FEMALE RATS.

Treatment ACTH IU/rat/day	Treatment Growth hormone and prolactin mg/rat/day						
	None	GH_5	GH_{10}	P_5	P_{10}	$GH_5 + P_5$	$GH_{10} + P_{10}$
				Weight—g/100 g body weight			
None	3.56 ±0.08*[a]	3.85 ±0.14**	4.54 ±0.06	3.85 ±0.20	3.86 ±0.11	4.22 ±0.23	4.13 ±0.21
4	4.89 ±0.08	5.40 ±0.20	—	4.71 ±0.20	—	5.23 ±0.13	—
12	4.94 ±0.13	5.62 ±0.05	—	4.88 ±0.08	—	5.57 ±0.13	—
				Total lipids mg/g			
None	71.2 ±4.52	76.6 ±3.30	69.1 ±3.57	62.6 ±2.62	68.2 ±5.27	67.1 ±3.78	64.7 ±1.5
4	104.0 ±6.47	121.1 ±5.90	—	88.0 ±4.92	—	93.3 ±8.18	—
12	117.3 ±6.04	129.1 ±15.55	—	94.1 ±4.55	—	94.2 ±5.84	—
				Phospholipids content—mg/100 g body weight			
None	115 ±3.8	127 ±4.8	153 ±6.6	156 ±9.8	—	140 ±6.7	139 ±13.8
4	132 ±5.7	193 ±7.9	—	153 ±7.9	—	171 ±17.3	—
12	150 ±7.8	194 ±9.1	—	148 ±12.2	—	180 ±4.6	—
				Adrenal gland weight—mg/100 g body weight			
None	29.48 ±1.17	27.10 ±1.06	33.21 ±0.49	28.08 ±0.69	27.72 ±0.47	43.55 ±8.00	34.66 ±0.80
4	81.53 ±7.47	82.12 ±4.79	—	76.40 ±6.28	—	85.50 ±7.05	—
12	181.00 ±10.9	146.00 ±4.94	—	186.00 ±18.65	—	128.00 ±4.63	—

[a] Mean ± SE; 5 rats per group; * = 12 observations; ** = 10 observations; Daily doses: GH_5 = 5 mg; GH_{10} = 10 mg; P_5 = prolactin, 5 mg; P_{10} = prolactin, 10 mg. Hormones were injected twice daily over 10 days.

prolactin. In addition, the results support the data reported by Bates *et al.* (6) that simultaneous application of GH, prolactin and ACTH reduces the effect of ACTH upon adrenal gland weight.

1. Baker, B. L., Ingle, D. J., Li, C. H., and Evans, H. M., Amer. J. Anat. **82**, 75 (1948).
2. Li, C. H., Ingle, D. J., Evans, H. M., Prestruda, M. C., and Nezamis, J. E., Proc. Soc. Exp. Biol. Med. **70**, 753 (1949).
3. Raben, M. S., and Hollenberg, C. H., J. Clin. Invest. **38**, 484 (1959).
4. Fain, J. N., Kovacev, V. P., and Scow, R. O., J. Biol. Chem. **240**, 3522 (1965).
5. Hollenberg, C. H., Vost, A., and Patten, R. L., Rec. Prog. Hormone Res. **26**, 463 (1970).
6. Bates, R. W., Milković, S., and Garrison, M. M., Endocrinology **74**, 714 (1964).
7. MacKay, E. M., Amer. J. Physiol. **120**, 361 (1937).
8. MacKay, E. M., and Barnes, R. H., Amer. J Physiol. **118**, 525 (1937).
9. Milković, S., Garrison, M. M., and Bates, R. W., Endocrinology **75**, 670 (1964).
10. Behrman, H. R., Orczyk, G. P., MacDonald, G. P., and Greep, R. O., Endocrinology **87**, 1251 (1970).
11. Goodbridge, A. G., and Ball, E. G., Biochemistry N. Y., **6**, 2334 (1967).

12. Chadwick, A., and Jordan, B. J., J. Endocrinol. **49**, 51 (1971).
13. Outhouse, E. L., Farbes, J. C., and Richmond, Ph.D., J. Lab. Clin. Med. **25**, 1157 (1940).
14. Levin, L., and Farber, R. K., Rec. Prog. Hormone Res. **7**, 399 (1952).
15. Greenbaum, A. L., and McLean, P., Biochem. J. **54**, 407 (1953).
16. Goodman, H. M., Endocrinology **81**, 1027 (1968).
17. McGarry, E. E., Rubinstein, D., and Beck, J. C., Ann. N. Y. Acad. Sci. **148**, 559 (1968).
18. Hamid, M. A., Rubinstein, D., Ferguson, K. A., and Beck, J. C., Biochem. Biophys. Acta **100**, 179 (1965).

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