media. T.C. 1066 is most nearly adequate, while White's solution may serve for brief studies. It seems, however, that additions to or modifications of these media will be necessary for complete maintenance and differentiation of amphibian gonads *in vitro*.

Summary. Ovaries and testes of second-year larvae of *Rana catesbeiana* were cultured on synthetic media for varying periods of time up to 28 days. The organs were not as well maintained as on media from natural sources, but proliferation of non-germinal cells and young germ cells occurred on some of the tested media.

1. Foote, C. L., Foote, F. M., *Anat. Rec.*, 1958, v130, 553.

2. Fell, H. B., Robison, R., *Biochem. J.*, 1929, v23, 767.

3. Morgan, F. F., Morton, H. J., Parker, R. C., PROC. SOC. EXP. BIOL. AND MED., 1950, v73, 1.

4. White, P. R., *The cultivation of animal and plant cells*, 1954, Chap. 5, N. Y., Ronald Press.

5. Shaffer, B. M., *Exp. Cell Res.*, 1956, v11, 244.

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Effect of Growth Hormone on Plasma Unesterified Fatty Acid Levels of Hypophysectomized Rats. (24173)

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Studies by Greenbaum *et al.*(1,2,3), Weil (4) and Curry(5) and others have shown that growth hormone effects mobilization of lipids from depots and probable utilization of these lipids as a primary source of energy for the body. The studies of Greenbaum(1) suggest that lipid mobilization and utilization are the probable mechanisms by which growth hormone effects positive nitrogen balance and hence growth. Russell(6) suggests that the "protein sparing" effect of growth hormone might be enhanced by furnishing large amounts of fat to the diet. Gordon(7,8) and Dole(9) postulate that unesterified fatty acid (UFA) is liberated from fat depots when other sources of calories are decreasing or are not available. This study relates the effect of growth hormone on plasma unesterified fatty acid levels of hypophysectomized rats. By using hypophysectomized animals it is possible to obviate the effect of endogenous growth hormone.

Methods. Female Sprague-Dawley rats hypophysectomized at 150 g (Exp. I and II) and 250 g (Exp. III) were obtained from Hormone Assay Inc., Chicago, Ill. Before the studies were undertaken the animals were kept on rat-chow plus meat supplement diet for 10-14 days post hypophysectomy. Animals whose body weight had plateaued were considered adequately hypophysectomized. On the morning the experimental animals were injected intraperitoneally with 4 mg of growth hormone [Somar-A (Armour R-50109) courtesy Endocrinology Study Section, USPHS] in 0.5 ml of distilled water adjusted to pH 9.5. Control rats in Exp. I were injected with 0.5 ml of distilled water adjusted to pH 9.5. In Exp. II and III one group of animals was injected with 4 mg of inactivated growth hormone in 0.5 ml distilled water adjusted to pH 9.5. The growth hormone was inactivated by autoclaving the solution at 18 lb, 123°C for 1 hour. The other group in these experiments was injected with an active growth hormone preparation which was from the same lot as the inactivated preparation. After injection, animals were placed in individual cages and provided *ad libitum* with drinking solution containing 10% dextrose in 0.5 normal saline. The animals were sacrificed by decapitation 8 hours after injection. This time interval was demonstrated by preliminary studies with fasted rats to be the time of most significant difference of UFA levels between experimental and control animals.

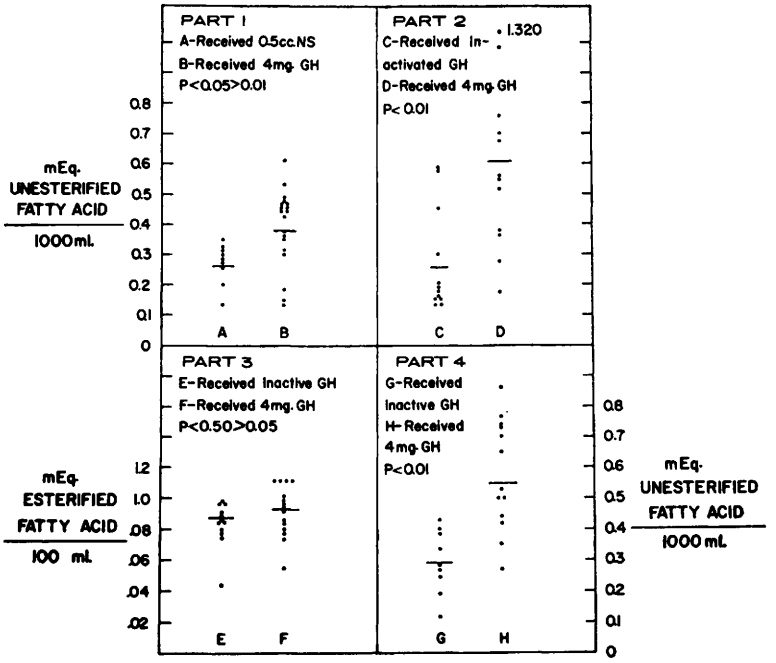

FIG. 1.

The decapitated animals were bled into centrifuge tubes containing Wintrobe's oxalate as anticoagulant. Unesterified fatty acids were determined by the method of Gordon(6) and esterified fatty acids by the method of Hack(10). A minimum of 1 ml of plasma was required for accurate determination of unesterified fatty acid levels.

Results. In Exp. I animals were injected with 4 mg of growth hormone, while controls received distilled water. The data of Fig. 1, Part 1 show that there was a significant difference in the plasma unesterified fatty acid levels between the 2 groups at the end of 8 hours. The mean of the experimental group was 0.392 meq UFA/1000 ml, while that of the control group was 0.265 meq UFA/1000 ml. This difference was significant at the 5% level but not at 1%. Exp. II was planned to determine the effect of injection of protein on serum unesterified fatty acid versus the effect of growth hormone injection. Therefore, in this experiment, 1 group of animals received inactivated growth hormone while the other received an identical amount of an active preparation. As shown in Fig. 1, Part 2, at the end of 8 hours the mean of the group receiving the active preparation was 0.605 meq UFA/1000 ml while that of the group receiving the inactive preparation was 0.258 meq UFA/1000 ml. This difference was significant at the 1% level. Exp. III was then planned to determine the effect of growth hormone on levels of serum esterified fatty acids and the relationship of these to serum levels of unesterified fatty acid. It was technically impossible to measure these levels sequentially. Fig. 1, Part 3 shows that the mean plasma esterified fatty acid concentration of the group receiving active growth hormone was 0.93 meq/100 ml after 8 hours, while that of the group receiving the inactivated preparation was 0.88 meq/100 ml. This difference is not significant at the 20% level. On the other hand, as can be seen from Fig. 1, Part 4, the mean of the unesterified fatty acid levels of the group receiving the active preparation was 0.566 meq UFA/1000 ml and that of the group receiving the inactive preparation was 0.294 meq UFA/1000 ml. This difference is significant at the 1% level.

Discussion. Our data would indicate that growth hormone has a definite effect on mo-

bilization of unesterified fatty acids in the hypophysectomized rat. Since the work of Gordon(7,8) and Dole(9) in humans indicates that with carbohydrate feeding the unesterified fatty acid level in the plasma decreases, it is of great interest to note that growth hormone caused an increase in plasma unesterified fatty acid in animals receiving carbohydrate *ad libitum*. The time required for this action to manifest itself (8 hours) correlates well with Greenbaum's(2) work in which a maximal increase in plasma and hepatic lipids occurred 6 hours after growth hormone injection. Weil's(11) work revealed a doubling of liver fat 7 hours post injection of 100 mg growth hormone.

At the present time the mechanism of growth hormone's mobilization of unesterified fatty acids from fat depots can be only speculated upon.

Release or formation of unesterified fatty acid following growth hormone administration could well provide an immediate source of energy, and thereby contribute to the protein anabolism seen after growth hormone administration. This phenomenon is well demonstrated by the studies of Greenbaum(1) and Beaton and Curry(4) who found that administration of growth hormone produced marked decrease in body adipose tissue and concomitant increase in body protein. The source of this unesterified fatty acid appears to be from adipose tissue according to studies by Gordon *et al.*(7). The observation has been made that serum esterified fatty acids are not altered by growth hormone administration and this may indicate that the unesterified fatty acid rise is derived *via* a different metabolic pathway.

Summary. Injection of growth hormone into hypophysectomized rats causes a significant rise in concentration of plasma unesterified fatty acids after an 8 hour interval. This rise in unesterified fatty acids is not associated with a concomitant rise in esterified fatty acid. Injection of inactivated growth hormone does not cause an increase in plasma unesterified fatty acid levels. It is postulated that this rise in unesterified fatty acids may provide an important source of energy for the known protein anabolic effect of growth hormone.

1. Greenbaum, A. L., *Biochem. J.*, 1953, v54, 400.
2. Greenbaum, A. L., McLean, P., *ibid.*, 1953, v54, 407.
3. ———, *ibid.*, 1953, v54, 413.
4. Weil, R., *Arch. Int. Med.*, 1955, v95, 739.
5. Beaton, G. H., Curry, D. M., *Endocrinology*, 1956, v58, #6, 797.
6. Russell, J. A., *Hypophyseal Growth Hormone Nature and Actions* (International Symposium, Blakiston, Detroit, 1955, 213.
7. Gordon, R. S., Cherkes, A., Gates, H., *J. Clin. Invest.*, 1957, vXXXVI, Part I, 810.
8. Gordon, R. S., Cherkes, A., *ibid.*, 1956, vXXXV #2, 206.
9. Dole, V. P., *ibid.*, 1956, v35, 150.
10. Hack, M., *Arch. Biochem. and Biophys.*, 1957, v58, 19.
11. Weil, R., Ross, S., *Endocrinology*, 1949, v45, 207.

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Occurrence of Fibrinolytic Activity Following Administration of Nicotinic Acid.* (24174)

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In vivo activation of fibrinolytic substances in blood has been accomplished by a variety of enzymes and extracts prepared from urine, plasma and tissues(1,2,3). This report describes a consistent observation of fibrinolytic activity in man following intravenous injection of a relatively simple substance, nicotinic acid. The observation was made during a

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