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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TABLE I. Effect of I.V. Nicotinic Acid (1.0 mg/K) in Man on Clotting and Fibrinolytic Activity. (75 mg as sodium salt in 7.5 ml inj. over a 4 min. period.)

Specimen	Time after completion of inj., min.	Prothrombin time (sec.) Whole plasma	Prothrombin time (sec.) Diluted (12.5%) plasma	Recalcification time, sec.	Residual thrombin activity,* sec.(7)	Plasma lysis† In 2 hr	Plasma lysis† In 24 hr	Coagulograph amplitude (mm) Maximum	Coagulograph amplitude (mm) At 2 hr
A	Control	14.5	28.5	63.0	12.5	0	0	53	53
B	0	14.5‡	28.0	85.5	12.0	0	0	51	51
C	1					0	0	36	36
D	2½					0	0	36	35
E	4					0	+	31	29
F	6½					±	++	18	3
G	10	15.0‡	29.0	50.0	12.0	+	++	35	3
H	13					++	++	29	1
I	16					±	++	23	23
J	20	15.0‡	30.5	47.0	11.0	0	++	44	43§
K	35	15.0	31.5	63.0	13.5	0	++	33	33§
L	Control + 50 mg/l nicotinic acid	14.5	30.0	58.5	10.5	0	0	39	39

* By the technic used, 10 to 16 sec. is normal range of residual thrombin activity, with longer times reflecting increased antithrombin activity, and shorter times decreased antithrombin activity(7).

† 0 = no lysis; ± = minimal lysis; + = moderate lysis; ++ = complete lysis.

‡ Clots which formed were noted to have a fragile quality when lifted with a wire loop, although the endpoint of clotting test was clear.

§ Markedly reduced 24 hr later.

study of the influence of vasodilator drugs on the clotting mechanism.

Results. Table I describes the results of a typical experiment. Plasma specimens obtained 4 to 35 minutes after intravenous injection of 1 mg/kg of nicotinic acid in a 67-year-old hypertensive female over 4 minutes, showed evidence of fibrinolytic activity both by direct observation of the recalcified specimen in a test tube at 37°C, and, more quantitatively, by the coagulograph ("Thrombelastograph" of Hartert(4)) instrument. Although the patient noted the nicotinic acid "flush" primarily during injection, the specimens drawn up to 4 minutes after completion of injection showed little or no fibrinolytic activity. A decrease in clot firmness was noted one minute after completion of injection, with definite fibrinolysis in the specimen drawn 4 minutes after injection. Maximum fibrinolytic activity occurred in 10 to 15 minutes. Clotting time reactions were not significantly altered.

Fig. 1 presents the coagulograph patterns obtained in this experiment. The vertical distance between pairs of lines recorded for each specimen is a function of the firmness of the clot at any given moment(5,6). The control specimen (A) demonstrates the typical normal pattern of recalcified human citrated plasma. A single straight line is recorded prior to clot formation. With increasing clot firmness, a pair of diverging lines are recorded

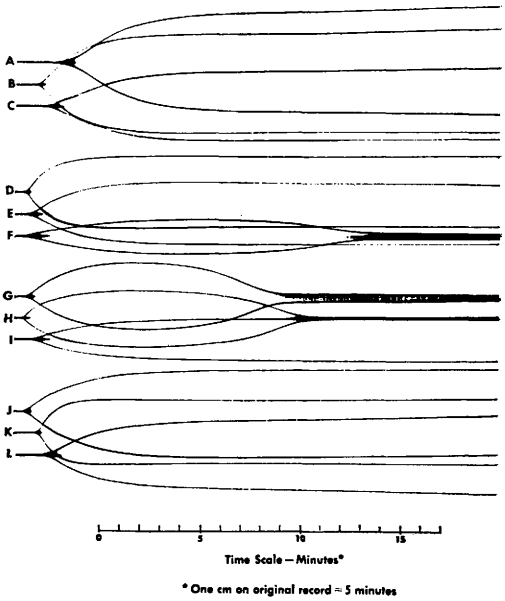

FIG. 1.

until maximum firmness is developed, at which time the lines become parallel. If lysis ensues, the lines begin to converge and merge into a single line when lysis is essentially complete. The pattern of lysis following nicotinic acid in this patient resembles that seen with lysis induced by material extracted from swine plasma(5) in contrast to the more gradual lysis seen with streptokinase activation. Specimen (L), which represents control plasma to which 50 mg/L of nicotinic acid had been added *in vitro,* shows no evidence of lysis. In other experiments, *in vitro* addition of nicotinic acid in concentrations ranging from 0.05 to 500 mg/liter plasma failed to influence fibrinolytic activity or clot firmness. In contrast, intravenous administration of nicotinic acid to 10 human subjects in doses of 1 to 3 mg/K induced fibrinolytic activity in all 10 instances. In this respect, the development of fibrinolytic activity by nicotinic acid *in vivo,* but not *in vitro,* resembles the activation of clearing factor by heparin.

Conclusion. It is concluded that a relatively simple non-enzymatic substance, nicotinic acid, can induce fibrinolytic activity *in vivo.*

1. Mullertz, S., Lassen, M., PROC. SOC. EXP. BIOL. AND MED., 1953, v82, 264.

2. Bierstedt, P., *Proc. I Internat. Conf. Thrombosis and Embolism,* p99, Benno Schwabe and Co., Basel, 1954.

3. Kaulla, K. N., von, *Nature,* London, 1949, v164, 40.

4. Hartert, H., *Z. ges. exp. Med.,* 1951, v117, 189.

5. Kaulla, K. N. von, Weiner, M., *Blood,* 1955, v10, 362.

6. Weiner, M., Weisberg, L. G., *Blood,* 1957, v12, 1125.

7. Weiner, M., "Residual Serum Thrombin Activity," Meeting of Soc. for Exp. Biol. and Med., at N. Y. Acad. Med., Mar. 28, 1956.

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Keratinization *in vitro* of chorionic Epithelium of the Chick Embryo.* (24175)

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The chorionic epithelium (CE) of the chick embryo forms the outer surface of the chorio-allantoic membrane (CAM). It consists of a single layer of small, attenuated cells of ectodermal origin, distally apposed to the shell-membrane and closely associated with blood vessels in the underlying mesenchyme in a manner characteristic of a respiratory epithelium (Fig. 1). Its basic structure is essentially retained also throughout later phases of embryonic development (Fig. 2), when capillaries penetrate through the CE to form a dense network on its surface. This chorio-vascular respiratory system continues to evolve until time of hatching when this structure, together with the rest of the CAM, is discarded with the shell. In its normal course of development, the CE shows no overt indications of morphogenetic propensities other than those which it displays in connection with its role as a respiratory epithelium; the possibility that its cells might possess additional potentialities which they would display under appropriate conditions, has not been adequately explored. In connection with another study, an attempt was made to maintain CE in organ culture in its original attenuated form. It was found, however, that the explants rapidly underwent striking metaplastic changes, during which they acquired new structural and functional characteristics.

Material and methods. Small fragments of CAM were excised from 8-day embryos, rinsed thoroughly in Tyrode's solution, then stretched on small squares of rayon-acetate net(1), the CE facing up, and cultured by

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