F. A. Davis Co., Philadelphia, 1965, p247.
5. Kraft, S. C., Rimpila, J. J., Fitch, F. W., Kirsner, J. B., Arch. Path. (Chicago), 1966, v82, 369.
6. Broberger, O., Perlmann, P., J. Exp. Med., 1962, v115, 13.
7. Koffler, D., Minkowitz, S., Rothman, W., Garlock, J., Am. J. Path., 1962, v41, 733.
8. Klavins, J. V., J.A.M.A., 1963, v183, 547.
9. Harrison, W. J., Lancet, 1965, v1, 1346.
10. Goldstein, G., Slizys, I. S., Chase, M. W., J. Exp. Med., 1961, v114, 89.
11. Goldstein, G., Spalding, B. H., Hunt, W. B., Jr., Proc. Soc. Exp. Biol. & Med., 1962, v111, 416.
12. Kraft, S. C., Rimpila, J. J., Fitch, F. W., Kirsner, J. B., Gastroenterology, 1964, v46, 747.
13. Wood, B. T., Thompson, S. H., Goldstein, G., J. Immunol., 1965, v95, 225.
14. Sainte-Marie, G., J. Histochem. Cytochem., 1962, v10, 250.
15. Coons, A. H., Kaplan, M. H., J. Exp. Med., 1950, v91, 1.
16. Mellors, R. C., Analytical Cytology, McGraw-Hill, New York, 1959, p9.
17. Goldwasser, R. A., Shepard, C. C., J. Immunol., 1958, v80, 122.
18. McKinney, R. M., Spillane, J. T., Pearce, G. W., ibid., 1964, v93, 232.
19. Hiramoto, R., J. Histochem. Cytochem., 1964, v12, 271.
20. Hultén, J., Pontén, J., Acta Path. Microbiol. Scand., 1964, v60, 1.
21. Myers, J., Sargent, A. U., Cohen, J. J., Immunology, 1965, v9, 101.
22. Broberger, O., Gastroenterology, 1964, v47, 229.

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Effect of Nicotinic Acid Administration on Plasma Growth Hormone Concentrations.* (32549)

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In recent years, it has been demonstrated that the secretion of human growth hormone (HGH) is markedly influenced by hypoglycemia(1), glucose(2,3) or amino acid(4) administration. In addition, other stimuli such as exercise, prolonged fasting or stress have been shown to augment plasma HGH(2,5). Similar to its various physiological effects, the secretion of HGH appears to be controlled by a variety of factors.

In this laboratory, an attempt was made in normal subjects, to induce plasma non-esterified fatty acids (NEFA) change by administration of nicotinic acid, with or without heparin injection, and to determine serial plasma HGH concentrations. This report describes our finding that marked increase of plasma HGH has occurred following the administration of nicotinic acid alone, and this increase was abolished when nicotinic acid was given in combination with heparin injection.

Materials and methods. All test subjects were healthy male students, their ages ranging from 21 to 24 years old. They were non-obese, and had no family history of diabetes mellitus. All had fasted overnight for 13-15 hours. Upon arrival at the hospital next morning, the subjects had bed rest for at least 30 minutes before starting the test. They were on bed rest and told to relax but not to sleep during the study.

Indwelling needle was inserted into the antecubital vein and slow infusion (approximately 10 drops per minute) of physiological saline was performed to prevent blood coagulation throughout the test. All injections and blood samplings were done through the indwelling needle.

Tests were divided into 3 groups as follows: (a) Nine cases received 20 ml saline at 0 and 20 minutes. (b) Five cases received 100 mg nicotinic acid in 20 ml saline at 0 and 20

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minutes. (c) Four cases received 100 mg nicotinic acid twice as above and 1000 units heparin at 10 minutes. Blood was drawn with heparinized syringes before and at appropriate intervals for 180 minutes as indicated in Figures. Plasma was obtained by immediate centrifugation and samples were stored at —20°C until the time of measurement.

Plasma HGH concentrations were determined by the method of Glick, Roth, Yalow and Berson(6). Raben HGH was used as antigen, iodinated HGH, and standard. The minimum detectable level of plasma HGH was 0.25 mμg/ml using 10-fold dilution of plasma sample. Plasma glucose and NEFA were measured by glucose oxidase method(7) and Dole's method(8).

Results. Results are shown in Figs. 1-3. For statistical analysis, plasma HGH value below 0.25 mμg/ml was calculated as equal to 0.25 mμg/ml.

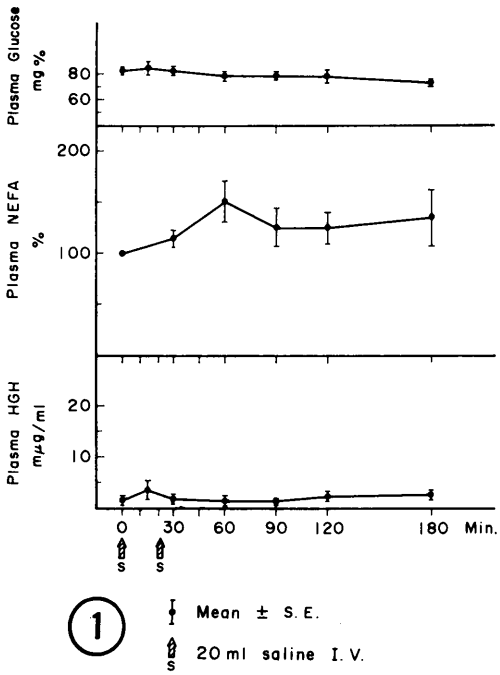
(1) In the control group (Fig. 1), there was no significant change of plasma glucose, plasma NEFA, and HGH during the 3-hour test period.

(2) In the group of nicotinic acid injection (Fig. 2), there was an initial decrease of plasma NEFA to approximately 60%, then a return to near baseline at 120 minutes, followed by a sharp rise at 180 minutes (secondary rise). Plasma glucose showed no significant changes.

Plasma HGH showed a marked rise at 120 minutes and/or at 180 minutes in all cases, and these values were significantly higher ($p<0.005$) than those of the control group.

(3) In the group of nicotinic acid and heparin injection (Fig. 3), plasma NEFA showed a slight initial rise, but did not show significant reduction below the fasting level. The secondary rise of NEFA was decreased. Plasma glucose and HGH showed no significant changes.

Discussion. In view of the fact that stress can cause a precipitous rise of plasma HGH (9,10), caution was exercised for the careful handling of the test subjects. Furthermore, to avoid the possible influence of sleep(11, 12) to the secretion of HGH, the subjects were prohibited from sleeping during the test.



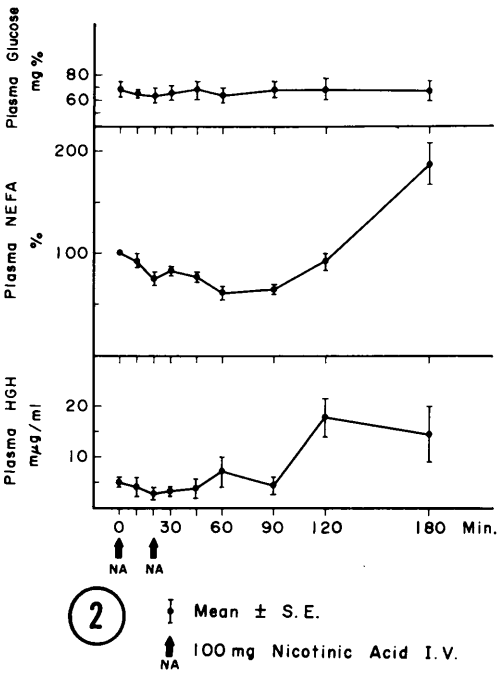


FIG. 1. Plasma glucose (mg %), NEFA (% of initial), and HGH (mμg/ml) following 20 ml saline injection at 0 and 20 min in 9 cases.

FIG. 2. Plasma glucose (mg %), NEFA (% of initial), and HGH (mμg/ml) following 100 mg nicotinic acid (in 20 ml saline) injection at 0 and 20 min in 5 cases.

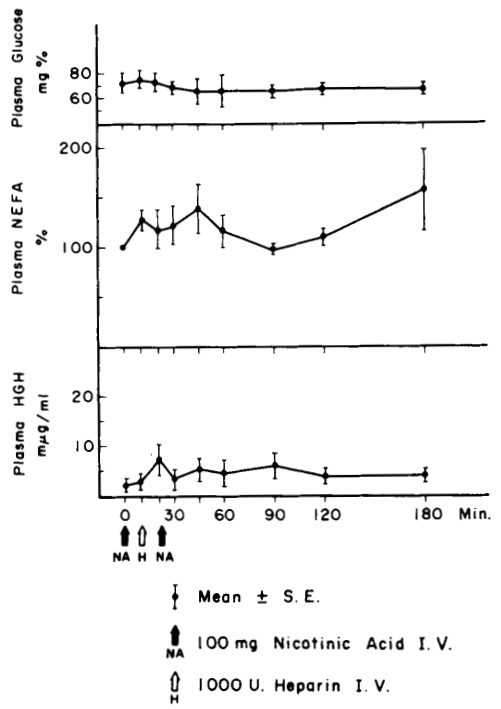


FIG. 3. Plasma glucose (mg %), NEFA (% of initial), and HGH (mμg/ml) following 100 mg nicotinic acid (in 20 ml saline) injection at 0 and 20 min and 1000 unit heparin injection at 10 min in 4 cases.

Under these circumstances, 9 control subjects, who received saline injections, showed no appreciable changes of plasma HGH during the 3-hour period. This is in accordance with the finding of Frohman *et al*(13). Recent observation by Pinter and Pattee(14) pointed out the biphasic change of blood glucose following 400 mg nicotinic acid infusion, but this effect was not observed in our experiment in which 100 mg nicotinic acid was injected twice. Administration of nicotinic acid alone or with the combination of heparin injection, caused the same degree of flushing of the face and extremities in most cases, and this was not of great discomfort to the subjects. Thus the increase in plasma HGH seen in the subjects of Group 2 was not considered due to stress or the vasodilating effect of nicotinic acid. Moreover, the increase of plasma HGH was not observed until 120 minutes in Group 2, probably denying the direct effect of nicotinic acid for this increase.

From these results it is suggested that: (a) lowering of plasma NEFA levels by nicotinic acid administration can stimulate the secretion of HGH, (b) this stimulation is abolished when the reduction of plasma NEFA levels was prevented by heparin injection. Since plasma glucose levels remained unchanged, and mechanisms of the plasma NEFA reduction by nicotinic acid(15,16), and its augmentation by heparin(17) are quite different, the stimulation of HGH appears to depend solely upon the levels of plasma NEFA.

This is particularly interesting in view of the well-known effect of HGH in stimulating lipolysis(18), namely, one could assume a negative feed-back mechanism between plasma NEFA and HGH. Preliminary results of the experiment of salicylate administration showed the reduction of plasma NEFA and the increase of plasma NEFA, the finding similar to the result of this experiment.

It is of interest to note that the augmentation of plasma HGH has been observed in the condition which causes the enhancement of lipolysis, such as exercise, prolonged fasting, stress or a 4-6 hour period following glucose administration(5). Under these conditions, either the existence of a common stimulatory factor of plasma HGH and NEFA or a possible contribution of intracellular NEFA deprivation to stimulate HGH secretion may be suggested. Carlson *et al*(19) have noted an initial drop of plasma NEFA level (circulatory phase) during moderate exercise in man. The reduction of plasma NEFA following glucose administration is well established(8). Insulin hypoglycemia(8) and amino acid administration(20), which are known to cause the stimulation of HGH secretion, are also known to cause the reduction of plasma NEFA. Whether the plasma NEFA plays a role in these situations is to be determined.

As shown in Fig. 2, the augmentation of plasma NEFA was preceded by that of plasma HGH. When heparin injection was combined (Fig. 3), the degree of secondary rise of NEFA decreased and the plasma HGH rise was not seen. Although further studies are needed, plasma HGH may, at least in

part, participate in inducing the secondary rise of plasma NEFA following the injection of nicotinic acid.

Summary. Serial plasma glucose, NEFA, and HGH concentrations were measured in normal male subjects following saline injection (Group 1, 9 cases), nicotinic acid injection (Group 2, 5 cases), and nicotinic acid plus heparin injection (Group 3, 4 cases) for 180 minutes. There was no appreciable change of plasma glucose in all groups. In Group 1 there was no significant change of plasma, NEFA and HGH. In Group 2, plasma NEFA showed an initial decrease followed by the secondary rise at 180 minutes, and plasma HGH showed a marked rise at 120 minutes and/or at 180 minutes. In Group 3, plasma NEFA did not show significant reduction and plasma HGH showed no significant changes. From the results obtained, it was suggested that the lowering of plasma NEFA levels by nicotinic acid administration can stimulate the secretion of HGH, and an assumption was made that plasma NEFA could be at least one of the factors in regulating HGH secretion. It was also suggested that plasma HGH may, at least in part, participate in inducing the secondary rise of plasma NEFA following the injection of nicotinic acid.

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1. Roth, J., Glick, S. M., Yalow, R. S., Berson, S. A., Science, 1963, v140, 987.
2. ———, Metabolism, 1963, v12, 577.
3 ———, Diabetes, 1964, v13, 355.
4. Knopf, R. F., Conn, J. W., Fajans, S. S., Floyd, J. C., Guntsche, E. M., Rull, J. A., J. Clin. Endocrinol., 1965, v25, 1140.
5. Glick, S. M., Roth, J., Yalow, R. S., Berson, S. A., Recent Progr. Hormone Res., 1965, v21, 241.
6. ———, Nature (London), 1963, v199, 784.
7. Huggett, A. St.G., Nixon, D. A., Lancet, 1957, v2, 369.
8. Dole, V. P., J. Clin. Invest., 1956, v35, 150.
9. Greenwood, F. C., Landon, J., Nature (London), 1966, v210, 540.
10. Meyer, V., Knobil, E., Endocrinology, 1967, v80, 163.
11. Hunter, W. M., Rigal, W. M., J. Endocrinol., 1966, v34, 147.
12. Quabbe, H.-J., Schilling, E., Helge, H., J. Clin. Endocrinol., 1966, v26, 1173.
13. Frohman, L. A., Horton, E. S., Lebovitz, H. E., Metabolism, 1967, v16, 57.
14. Pinter, E. J., Pattee, C. J., J. Clin. Endocrinol., 1967, v27, 440.
15. Carlson, L. A., Orö, L., Acta Med. Scand., 1962, v172, 641.
16. Carlson, L. A., ibid., 1963, v173, 719.
17. Korn, E. D., J. Biol. Chem., 1955, v215, 15.
18. Raben, M. S., Hollenberg, C. H., J. Clin. Invest., 1959, v38, 484.
19. Carlson, L. A., Ekelund, L. G., Orö, L., J. Lab. Clin. Med., 1963, v61, 724.
20. Floyd, J. C., Jr., Fajans, S. S., Conn, J. W., Knopf, R. F., Rull, J., J. Clin. Invest., 1966, v45, 1487.

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Inhibition of the Lymphopenic Effect of Cortisol by Puromycin Injection in Mice.* (32550)

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The lymphopenic action of adrenal cortical steroids was established a number of years ago, and is well documented(1,2). At 3 to 6 hours following a single injection of an effective steroid, blood lymphocytes values are depressed to a minimum. Stimulation of the pituitary-adrenal axis by any one of a wide

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