TABLE VII. Tumor Inhibiting Effect of Relatively High Concentrations of NaF Added to Tumor Suspensions Before Implantation into Eggs.

No. of experiments	No. eggs Control	No. eggs Exp	Dosage, mg/.2 ml injection	Tumor wt, control = 100
4	63	58	.1	70
4	62	65	.2	51
3	37	35	.4	42
6	78	83	.8	6
Totals: 17	240	241		

Control avg tumor wt (g) .39.
Exp avg tumor wt (g) .14.

in dosage increased tumor growth in the instance of both NaF and NaBr. It was recently reported(5) that the plasma fluoride is regulated in rats. This probably accounts for the similarity of tumor growth acceleration when NaBr is given over a range of 1 to 1,000 mg per liter, and NaF in concentrations of 1 to 55 mg per liter.

Unpublished results of tests with NaI indicate that this halide also stimulates tumor growth when added to tumor suspensions before implantation in eggs or mice at low levels, 0.000,1-0.001 mg per mouse or egg.

Summary. In 54 tests involving 991 mice bearing transplanted tumors and 58 tests including 1817 tumor-bearing eggs, data were obtained which indicated a statistically significant acceleration of tumor tissue growth in association with comparatively low levels of NaF.

1. Taylor, A., Taylor, N. C., Cancer Research, 1964, v24, 751.
2. Taylor, A., Carmichael, N., Norris, T., ibid., 1948, v8, 264.
3. Taylor, A., Univ. Texas Publication, 1953, n5314, 13.
4. ———, ibid., 1953, n5314, 27.
5. Singer, L., Armstrong, W. D., Proc. Soc. Exp. Biol. and Med., 1964, v117, 686.

Received February 9, 1965. P.S.E.B.M., 1965, v119.

Effect of Adrenalectomy on Hyperglycemic Responses of Fed and Fasted Rats to Catecholamines.* (30151)

ALEXANDER D. KENNY AND WILLIAM W. FLEMING (Introduced by D. T. Watts)
Department of Pharmacology, West Virginia University Medical Center, Morgantown

The hyperglycemic response to epinephrine has been difficult to fit into the classification of adrenergic receptors proposed by Ahlquist (1,2). The problem has been considerably resolved, at least in the case of the rat, by evidence that liver glycogenolysis is mediated by an α-receptor and that muscle glycogenolysis is mediated by a β-receptor(3-6). Fleming and Kenny(7) have demonstrated that in *fed* rats the hyperglycemic response has the characteristics of an α-response and in *fasted* rats of a β-response. These investigators pointed out that α-responses are best studied in the *fed* rat using norepinephrine and β-responses in the *fasted* rat using isoproterenol.

Adrenalectomy causes changes in the effects of epinephrine on carbohydrate metabolism (8,9). In view of the new evidence concerning the importance of the prandial state of rats and a greater understanding of the receptors involved in the hyperglycemic response, it was considered important to re-evaluate the effect of adrenalectomy using dose-response curves and both *fed* and *fasted* animals.

Materials and methods. Blood glucose experiments. Male Holtzman rats, from 6 to 8 weeks old, were maintained on a commercial laboratory diet throughout the study. When the rats were *fasted,* food was withheld during a period of from 16 to 20 hours before the beginning of the experiment. All rats, whether *fed* or *fasted,* were bled in the morn-

* This investigation was supported by USPHS research grants AM-04276 and AM-06544 from Nat. Inst. of Arthritis and Metab. Dis.; NB-03034 from Nat. Inst. of Neurol. Dis. and Blindness; and training grant, 5TI-GM-76, from Division of General Medical Sciences, USPHS.

ing. The animals were lightly anesthetized with ether and either a catecholamine or saline containing ascorbic acid was administered subcutaneously. One hour after the injection the rats were bled by cardiac puncture under light ether anesthesia. Blood glucose concentrations were determined by the method of Nelson(10). The effect of ether, as used in this procedure, on the experimental results has been shown to be negligible(7, 11). Rats were adrenalectomized an average of about 14 days before use by the technique described by Ingle and Griffith(12). Postoperatively the rats were given no special treatment except for addition of 1% sodium chloride to their drinking water.

Muscle glycogen experiments. The rats were treated as in the blood glucose experiments except that one hour after the injection each rat was lightly anesthetized with ether and approximately one gram of muscle was removed from the lateral surface of the thigh and quickly placed in 4.0 ml of 30% potassium hydroxide solution in a weighed, 25 ml, stoppered test tube. Glycogen was determined by the method of Good, Kramer and Somogyi(13). The results were expressed in mg of glucose/g of wet tissue.

Results. Fig. 1 shows that adrenalectomy did not significantly alter the hyperglycemic response of *fed* rats to norepinephrine ($0.1 > P > 0.05$ at the 5.0 mg/kg dose; $P = 0.2$ at the 0.5 mg/kg dose). In contrast, adrenalectomy considerably augmented the hyperglycemic response to isoproterenol in *fasted* rats (Fig. 2; $P < 0.05$ at all doses of 0.01 mg/kg and above, except 0.02 mg/kg). The enhanced action of isoproterenol on adrenalectomized *fasted* animals is also seen in reference to depletion of muscle glycogen (Fig. 3: $P < 0.05$ at all doses).

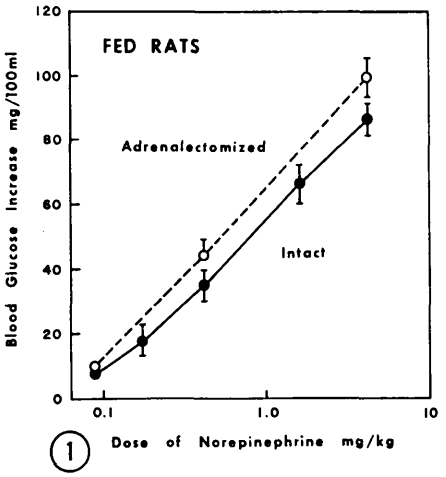
The effect of adrenalectomy on the hyperglycemic response to epinephrine in *fasted* rats was more complex (Fig. 4). In the lower doses, the action of epinephrine was enhanced by adrenalectomy; in higher doses, the effect was depressed. The differences were significant at all doses ($P < 0.05$) except 0.01 mg/kg.

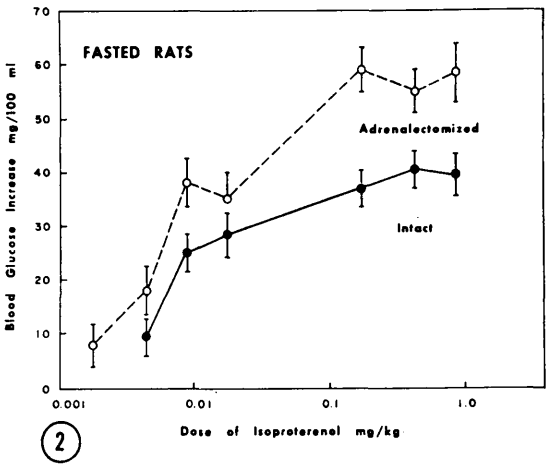
Discussion. Previous work has indicated that, in the rat, liver glycogenolysis in response to catecholamines is an α-response and muscle glycogenolysis is a β-response(3-6). In *fed* rats, the hyperglycemic response has the characteristics of an α-response, the blood glucose apparently coming from liver glycogenolysis; in *fasted* rats the hyperglycemic response has the characteristics of a β-response, the blood glucose apparently coming ultimately from muscle glycogenolysis(7). The ideal conditions for studying the α- and β-hyperglycemic responses respectively include norepinephrine in *fed* rats and isoproterenol in *fasted* rats(7). Based upon these criteria, the present results demonstrate that adrenalectomy enchances the β-hyperglycemic response but not that mediated by α-receptors. The increased hyperglycemic response can be explained on the basis of the increased depletion of muscle glycogen in the adrenalectomized animals since muscle glycogen is one of the ultimate sources of blood glucose in fasted animals. The increased glycogen-depleting effect of epinephrine in muscle of adrenalectomized *fasted* rats has been shown by Winternitz and Long(8). The greater loss of muscle glycogen in adrenalectomized rats is due to an inability to reconvert the lactic acid produced into glycogen, an effect which can be reversed by administration of hydrocortisone(15). The effect of adrenalectomy is thus the result of loss of the adrenal cortex rather than the medulla. The enhanced hyperglycemic response is probably the consequence of greater conversion of lactate to glucose resulting from higher blood levels of lactate since, unlike the conversion to glycogen, the conversion of lactate to glucose is not impaired by adrenalectomy(15).

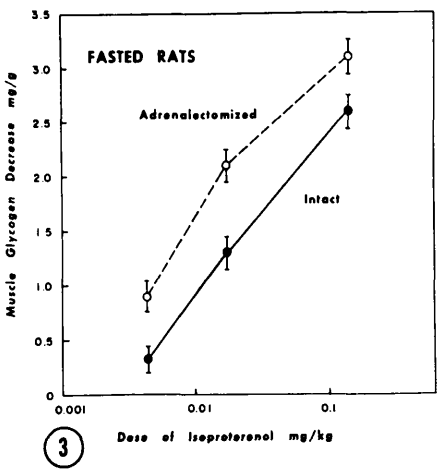
The action of epinephrine is more complex, due to the fact that this amine acts on both α- and β-receptors and thus on both liver and muscle glycogen. In the rat *fasted* for 24 hours, the glycogen content of the liver is reduced to 1% of control(14). In the lower dose range epinephrine must produce hyperglycemia in *fasted* rats almost entirely through muscle glycogenolysis and this effect is potentiated by adrenalectomy(8). On the basis of their results, Fleming and Kenny(7) suggested that in the higher dose range, the

hyperglycemic effect of epinephrine in intact *fasted* animals is due to both muscle and liver glycogenolysis, the liver glycogen arising *via* the Cori cycle(16) from the high level of lactate being released from muscle. This conclusion is strengthened by the present results. The hyperglycemic responses to higher doses of epinephrine are depressed by adrenalectomy and it has been shown(15) that adrenalectomized rats are unable to convert lactate to liver glycogen. Thus, while the epinephrine dose-response curve continues to rise in control *fasted* animals, the curve levels off at a low maximum in adrenalectomized *fasted* animals.

Summary. Adrenalectomy in rats has been shown to enhance the hyperglycemic response mediated by the β-receptor (isoproterenol in







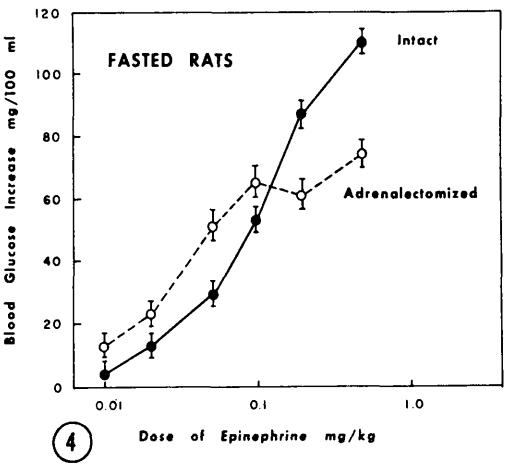


FIG. 1. Dose-response curves of hyperglycemic response of adrenalectomized *fed* rats and intact *fed* rats to norepinephrine. Each point represents mean of 14 to 15 rats. Bars indicate standard errors.

FIG. 2. Dose-response curves of hyperglycemic response of adrenalectomized *fasted* rats and intact *fasted* rats to isoproterenol. Each point represents mean of 10 to 18 rats. Bars indicate standard errors.

FIG. 3. Dose-response curves of the glycogen depletion in muscle of adrenalectomized *fasted* rats and intact *fasted* rats in response to isoproterenol. Each point represents mean of 14 to 17 rats. Bars indicate standard errors.

FIG. 4. Dose-response curves of hyperglycemic response of adrenalectomized *fasted* rats and intact *fasted* rats to epinephrine. Each point represents mean of 12 to 16 rats. Bars indicate standard errors.

the *fasted* rat) but not that mediated by the α-receptor (norepinephrine in the *fed* rat). The enhanced β-effect is apparently the result of a greater depletion of muscle glycogen. Hyperglycemic responses to epinephrine in *fasted* animals are enhanced in the lower dose range and depressed at higher doses. This is explained in terms of the effect of epinephrine on both α- and β-receptors. The importance of using both *fed* and *fasted* rats and dose-response curves rather than single doses in assessing the effects of adrenalectomy is demonstrated.

1. Ahlquist, R. P., Am. J. Physiol., 1948, v153, 586.
2. Furchgott, R. F., Pharmacol. Rev., 1959, v11, 429.
3. Vrij, C. J., Gho, B. K., Groot, C. A. de, Weber, J. F., Acta Physiol. Pharmacol. Neerl., 1956, v4, 547.
4. Pol, M. C. van der, ibid., 1956, v4, 541.
5. Hynie, S. von, Wenke, M., Muhlbachova, E., Arzneimittel-Forsch., 1961, v11, 858.
6. Roy, F. P. van, Schulhof, L. W., Arch. Int. Pharmacodyn., 1961, v130, 368.
7. Fleming, W. W., Kenny, A. D., Brit. J. Pharmacol., 1964, v22, 267.
8. Winternitz, W. W., Long, C. N. H., Proc. Soc. Exp. Biol. and Med., 1952, v81, 683.
9. Bloom, W. L., Russell, J. A., Am. J. Physiol., 1955, v183, 356.
10. Nelson, N., J. Biol. Chem., 1944, v153, 375.
11. Kenny, A. D., Morey, E. R., J. Pharmacol. Exp. Therap., 1962, v135, 317.
12. Ingle, D. J., Griffith, J. Q., The Rat in Laboratory Investigation, E. J. Farris and J. Q. Griffith, ed., J. B. Lippincott, Philadelphia, 1949, p444.
13. Good, C. A., Kramer, H., Somogyi, M., J. Biol. Chem., 1933, v100, 485.
14. Long, C. N. H., Katzin, B., Fry, E. G., Endocrinology, 1940 v26, 309.
15. Winternitz, W. W., Dintzis, R., Long, C. N., ibid., 1957, v61, 724.
16. Cori, C. F., Cori, G. T., J. Biol. Chem., 1929, v81, 389.

Received February 10, 1965. P.S.E.B.M., 1965, v119.

Effects of a Synthetic Analogue of Vasopressin on Vascular Smooth Muscle.* (30152)

B. M. ALTURA, S. G. HERSHEY AND B. W. ZWEIFACH
Departments of Anesthesiology and Pathology, New York University Medical Center, New York City

PLV-2, (2-phenylalanine-8-lysine vasopressin) a synthetic analogue of vasopressin has been reported to increase survival of rats subjected to hemorrhage(1,2). Direct *in vivo* microscopy of the microcirculation in the mesoappendix of rats in hemorrhagic shock (3) indicated that PLV-2 maintained arteriolar and venular tone, sustained vasomotion of metarterioles and precapillaries and decreased the abnormally high reactivity to topically applied epinephrine characteristic of shock. The net influence of the above selective effects of PLV-2 was to improve true capillary inflow, distribution and outflow. In view of the foregoing observations in the shocked animal, the effects of PLV-2† on microvascular smooth muscle of normal intact rats and on isolated arterial muscle strip preparations were studied.

Materials and methods. 1. *In vivo.* Wistar strain female rats, 130-180 g, were anesthetized with pentobarbital sodium, 3.0 mg/100 g body wt i.m. The mesoappendix was exteriorized for direct microscopic visualization according to a modification of a previously described method(4). Microscopic observations were made at magnifications of 60× and 100×. The effects of various concentrations of PLV-2 (Tables I, II) on the microvessels were determined: A. By direct (topical) application. B. By i.v. injection followed by observation of the vascular responses to standard concentrations of epi-

* Study aided in part by USPHS grants HE-09042-02 and HE-07289-02.

† The authors are indebted to Dr. R. Bircher, Sandoz Laboratories, for generously providing the PLV-2 used in these studies.