group next, and the glucagon group last. The behavior observed during the immediate postsurgical period was the result of the interaction of hormone treatment and the LH lesion, since hormone administration per se does not cause aphagia (*4, 7*).

The effect of prior hormone treatment on post-LH lesion body weight paralleled the feeding effect. Body weight differed across groups ($P < .001$; $F = 13.2$; d.f. = 2, 18) and across days ($P < .001$; $F = 4.6$; d.f. = 9, 162).

The results obtained were caused by the hormone treatment rather than by differences in the brain lesions. Histological examination of coronal sections revealed no major differences among groups (Fig. 2). These lesions are indistinguishable in size and location from those reported elsewhere to induce aphagia (*2, 3, 8*). The section in Fig. 2a was taken from a saline-treated animal that recovered eating behavior in 3 days. Parts b and c in Fig. 2 are essentially indistinguishable; Fig. 2b is from an insulin-treated animal with less than 1 day of aphagia, and Fig. 2c is from a glucagon-treated animal with 6 days of aphagia.

It has been hypothesized that the shortening of the LH recovery period produced by body weight reduction before surgery is due to a shift in a regulatory set point for body weight (*3*). However, we have demonstrated this phenomenon without altering body weight or food intake before surgery. The effect of the two glucodynamic hormones on the recovery period could be indirectly caused by their action on glucose utilization (*9*) or could be caused by their effects on neurotransmitters.

Reduction of brain norepinephrine by systemic injection of α-methyl-*p*-tyrosine 3 days before LH lesions reduces the recovery period (*10*), probably as a result of denervation supersensitivity. Norepinephrine has been hypothesized to be the neurotransmitter in the neural mediation of feeding behavior (*11*). Little is known of the effects of glucagon on the central nervous system. Insulin, however, has been shown to alter the levels of both tyrosine hydroxylase and dopamine β-hydroxylase (*12*), which are important enzymes in the conversion of tyrosine to norepinephrine. Perhaps, presurgical treatment with insulin, as with α-methyl-*p*-tyrosine, produces denervation supersensitivity in norepinephrine neural systems. Glucagon may also influence the norepinephrine system, but even less is known of its mode of action. However, insulin and glucagon may be influencing the postsurgical recovery in an entirely different manner.

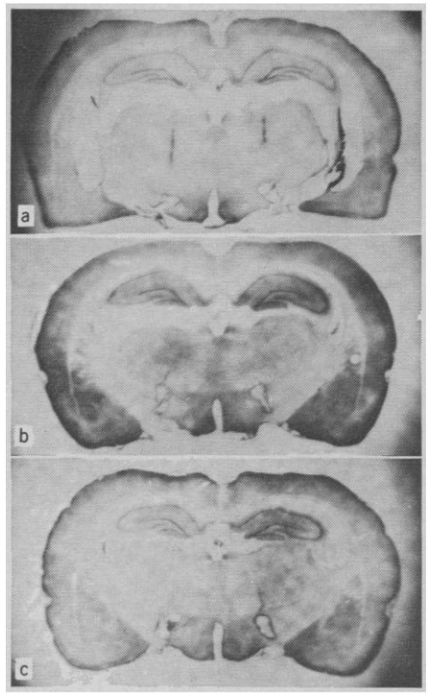

Fig. 2. Coronal sections passing through the midhypothalamus of rats treated with saline (a), insulin (b), or glucagon (c) before surgery. In every case the lesion encompassed the lateral hypothalamus, the inner edge of the internal capsule, and the ventral aspect of the fields of Forel.

Several structural and functional similarities have been observed between insulin and another protein, nerve growth factor (*13*). With this in mind, we would predict that prior administration of nerve growth factor to animals with LH lesions would also shorten the recovery period.

Regardless of the mechanism by which insulin and glucagon alter the recovery period, basically identical lesions to the same brain area in the present experiment led to three quantitatively different recovery periods. This type of phenomenon may underlie the recovery of function after damage to other parts of the central nervous system.

SAUL BALAGURA
LINDY HARRELL
TIMOTHY RALPH*
Department of Psychology, University of Massachusetts, Amherst 01002

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5. A dose of glucagon comparable to that used in this experiment has been shown to modify the rat's metabolism without greatly altering its caloric intake [J. M. Salter, *Amer. J. Clin. Nutr.* **8**, 535 (1960)]. In our experiment, glucagon-, insulin-, and saline-injected animals consumed all of their allotted food.
6. Lesions of the LH produce aphagia ranging from 3 days [1 ma for 7 seconds (*3*)] to seven or more days [2 ma for 30 seconds (*10*); 2 ma for 10 seconds (*8*)]. The lesion variables in our experiment were calculated to produce about 4 days of aphagia.
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* Present address: C. W. Post Center, Long Island University, Greenvale, N.Y. 11548.

2 January 1973; revised 9 April 1973

Dopa and Dopamine in Glusulase: Possible Artifact in Studies on Catecholamine Metabolism

Abstract. *Relatively high concentrations of dopa and dopamine were found in Glusulase, an enzyme preparation widely used in studies on catecholamine metabolism. This contamination may be a source of error in some studies, particularly in those measuring the endogenous concentrations of these catechols and their metabolic products.*

Recently, we reported a marked discrepancy between the plasma concentration of dopamine and its cardiovascular effects in man and dog (*1*). We considered the possibility that the major fraction of circulating dopamine might be an acid-labile, biologically inactive conjugate that was inadvertently hydrolyzed during its determination. Our analytical procedure for dopamine in-

volves perchloric acid precipitation of protein and elution of the catecholamines from Al_2O_3 with HCl (*2*). Small amounts of conjugated norepinephrine (0.5 ng/ml) and dopamine (3 ng/ml) have been reported in normal human plasma (*3*). Acid hydrolysis of plasma samples from several patients and dogs receiving dopa resulted in about a 20-fold and 3-fold increase, respectively, in dopamine concentration. An attempt was made to identify the conjugate by differential enzymatic hydrolysis with Glusulase, which contains sulfatase and β-glucuronidase, and Ketodase, which contains only β-glucuronidase. Unexpectedly, Glusulase-treated plasma from patients not receiving dopa yielded dopa and dopamine concentrations of about 1 μg/ml. This was a surprising finding in that free dopa and dopamine are undetectable (less than 1 ng/ml) in normal human plasma and conjugated forms have been reported at a concentration of about 3 ng/ml (*2*, *3*). Similar results were not obtained with the Ketodase-treated plasma, suggesting that the conjugate was a sulfate. Further studies, however, revealed that the Glusulase itself was the source of the catechols (Table 1).

In addition, as indicated in Table 1, the catechol content of the Glusulase is not the same in all batches of enzymes. Apparently the older preparations contain a lower concentration of catechols, with the dopa disappearing at a faster rate than the dopamine. In Fig. 1 we compare the fluorescence spectra of one of the Al_2O_3 extracts from Glusulase with the spectra of authentic dopa and dopamine. The extent of the acid-induced decrease in fluorescence is a reflection of the relative amounts of dopa and dopamine in the extract. Paper chromatographic comparison of a concentrate from several extracts with authentic dopa and dopamine confirmed their presence in the enzyme preparation. On the basis of the results from incubations of Glusulase with *p*-nitrophenyl glucuronide and *p*-nitrophenyl sulfate, there was no correlation between the hydrolytic activity of the Glusulase preparations and their catechol content. We did not detect norepinephrine or epinephrine in samples of Glusulase or Ketodase (less than 0.1 μg/ml).

Glusulase is Endo Laboratories' preparation of the crude intestinal juice of the snail, *Helix pomatia*. It has been widely used in studies on catecholamine metabolism since the time when it was

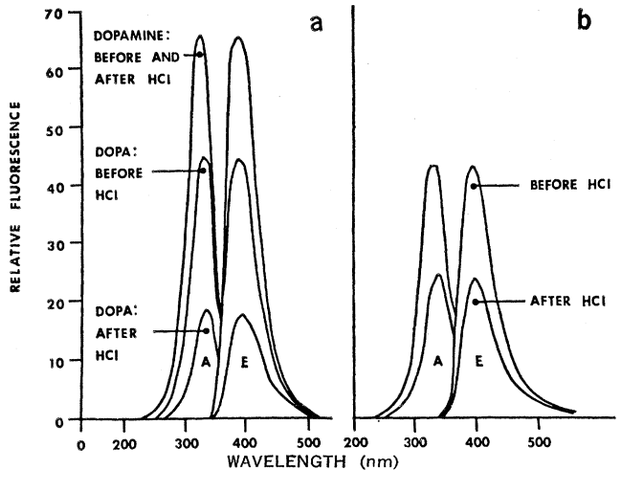

Fig. 1. (a) Fluorescence spectra for authentic dopamine and dopa before and after acidification with HCl; *A*, activation spectrum; *E*, emission spectrum. (b) Fluorescence spectra for the Al_2O_3 extract from Glusulase before and after acidification with HCl; *A*, activation spectrum; *E*, emission spectrum.

first reported for this purpose by Axelrod and colleagues in 1959 (*4*). However, insofar as we could determine, no mention has been made of results from a control experiment containing only Glusulase in an incubation. The usual procedure is to analyze biological material, such as urine, before and after an incubation (about 16 hours) with Glusulase, attributing any differences to the presence of a conjugate. It is a distinct possibility that during such long incubations, some of the dopa and dopamine in the Glusulase could be converted into catechol metabolites that might have been mistakenly attributed to metabolic activity in man and animals. Weil-Malherbe (*5*) has discussed the advantages and disadvantages of using these enzymes in the analysis of catecholamines, but he does not mention their catecholamine content. To what extent the presence of dopa and dopamine in an enzyme being used to study the products of these substances might change reported results cannot be said. However, in studies involving endogenous metabolites, this contamination could lead to a completely erroneous conclusion. It is interesting that dopamine has been found in the neural tissue of snails, including *Helix pomatia*. Kerkut *et al.* (*6*) reported relatively high concentrations of dopamine (5.5 μg/g) in the circumesophageal ganglion of *Helix aspersa*. This is the likely source of the catechol contamination in Glusulase since, anatomically, this ganglion is in very close proximity to the crop from which the Glusulase-containing intestinal juice is obtained. These investigators also found relatively high concentrations of dopa (0.18 μg/ml) in the blood of this snail.

Table 1. Dopa and dopamine in Glusulase. Portions of the enzyme preparations were analyzed for catecholamines (*2*) without prior incubation or hydrolysis. Incubation or acid hydrolysis did not increase the yield of dopa and dopamine. The batch numbers represent separate batches of Glusulase obtained at different times from Endo Laboratories. Batches 3 and 4 were borrowed from colleagues and were the oldest of the preparations. Batches 5 and 6 had the same lot number.

Batch number	Dopa (μg/100 ml)	Dopamine (μg/100 ml)
1	91	93
2	166	86
3	10	55
4	61	50
5	141	67
6	141	79

A. H. ANTON
A. SERRANO
Departments of Anesthesiology and *Pharmacology, Case Western Reserve School of Medicine, Cleveland, Ohio 44106*
T. B. TJANDRAMAGA
L. I. GOLDBERG
Clinical Pharmacology Program, Departments of Medicine and *Pharmacology, Emory School of Medicine, Atlanta, Georgia 30322*

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7. Supported in part by PHS grants GM-14270 and GM-06491. We thank A. R. Rohlik for technical assistance. Requests for reprints should be sent to A.H.A.

8 June 1973; revised 12 July 1973