brown fat suggests a unique role for this tissue in the sexual cycle of the bat.

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Effect of Histamine Liberators on Urinary Excretion of 5-Hydroxyindolacetic Acid.*† (26183)

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Bhattacharya and Lewis showed(1) that, in addition to histamine, serotonin is released from rat tissues perfused with Compound 48/80, a potent histamine liberator. Parratt & West(2,3) demonstrated that the anaphylactoid reaction induced in rats by dextran or egg white is chiefly mediated by release of serotonin. Further to these observations, a study was undertaken to evaluate urinary excretion of 5 - hydroxyindolacetic acid (5-HIAA) in rats receiving various histamine liberators. The results reported here show that the output of the serotonin metabolite is unchanged in animals treated with histamine liberators, even after repeated administration. When rats were made resistant to anaphylactoid edema by prolonged treatment with Compound 48/80, they still excreted as much 5-HIAA after injection of reserpine as did the untreated controls.

Materials and methods. Female rats of the Holtzman strain, weighing between 120 and 150 g, were used in these experiments. Twenty-four-hour urine specimens were collected in metabolic cages. During that time, animals were not fed but were adequately hydrated by gastric instillation of 2 ml of tap water 4 times daily. To keep urinary pH sufficiently low, a small amount of acetic acid was added to the flasks. Average urinary output was 10 ml and half of this volume was used for determination of 5-HIAA, following the method of Pierce(4).

In the first experiment, histamine liberators were injected once, at doses indicated in Table I, immediately before animals were caged. *In the second experiment,* Compound 48/80 was administered during 8 days in increasing doses ranging from 200 γ to 1 mg, as reported elsewhere(5), in order to desensitize animals to anaphylactoid edema. In fact, these rats manifested resistance to dextran (6 mg i.p.) and polymyxin B (1 mg s.c.). On the ninth day, they were challenged with 0.5 mg of reserpine, subcutaneously, and placed in metabolic cages for collection of urine.

Results. The values for urinary 5-HIAA are expressed in the Tables as μg/ml. Esti-

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TABLE I. Urinary Excretion of 5-HIAA in Rats Treated with Histamine Liberators.*

Groups of 6 rats	Dose, mg/day	Urinary 5-HIAA, μg/ml	"P" value relative to controls	Urine output, ml/24 hr
Controls	—	5.2 ± .21†	—	12.2 ± .45†
Compound 48/80	.25 × 2 s.c.	5.0 ± .51	>.5	12.3 ± 1.14
Polymyxin B	1. s.c.	5.3 ± .71	>.5	10.7 ± .97
Dextran	12. i.p.	5.8 ± .09	>.05	10.6 ± .87
Serotonin	.5 × 2 s.c.	19.1 ± 1.64		11.0 ± .84

* Animals were edematous during 6 hr. † Mean ± S.E.

mation of 24 hour values is obtained by multiplying these figures by total volume of urine excreted. Table I reveals that there are no significant differences between control rats and those treated with histamine liberators. Prolonged treatment with Compound 48/80 did not further modify excretion of 5-HIAA (Table II). In addition, it can be seen that animals so treated, although resistant to anaphylactoid reaction, still excreted as much 5-HIAA after reserpine injection as did non-pretreated rats.

Discussion. The ineffectiveness of histamine liberators to increase urinary excretion of 5-HIAA does not substantiate the claim that such liberators release serotonin. Failure to obtain this evidence may be ascribed to the fact that the effects of histamine liberators in living animals differ from those observed in perfusion experiments reported by Bhattacharya and Lewis(1). The optimal dose tolerated *in vivo* is perhaps inadequate to release sufficient tissue serotonin and raise urinary 5-HIAA. The studies of Lecomte seem quite relevant in this respect. It was observed that urinary 5-HIAA augments during anaphylaxis only in those animals which exhibit a severe state of shock(6,7).

TABLE II. Effect of Reserpine upon Urinary Excretion of 5-HIAA in Rats after Prolonged Treatment with Compound 48/80.

Groups of 12 rats	Urinary 5-HIAA, μg/ml	"P" value	Urine output, ml/24 hr
Controls	4.8 ± .32‡	.2	10.8 ± .43‡
Compound 48/80*	5.4 ± .38		12. ± .59
Reserpine†	9.4 ± .39	.4	7.75± .34
Compound 48/80* + reserpine† (0.5 mg s.c.)	10.6 ± .57		9.6 ±1.20

* Inj. s.c. during 7 days in increasing doses of 0.1 to 0.5 mg, twice daily.
† 0.5 mg inj. once s.c. following treatment with Compound 48/80.
‡ Mean ± S.E.

The present experiments also provide no support to the hypothesis that serotonin is the chief mediator of the anaphylactoid reaction. This is evidenced by the fact that rats made resistant to anaphylactoid edema react normally to the depleting action of reserpine on tissue serotonin. It may be assumed that Compound 48/80 and reserpine have 2 different sites of depleting action in rats but, should this be the case, the role of serotonin in the genesis of the cutaneous edema remains unexplained. In addition, one normally expects that serotonin liberation that occurs after reserpine treatment would give rise to anaphylactoid edema, and this is not the case. Reserpine, on the other hand, when given in sufficiently high doses, *i.e.*, 10 mg/kg, was shown by Parratt and West to desensitize rats to edema-producing agents(2). Following this treatment, animals become lethargic and do not eat or drink. However, the concurrent treatment with a central stimulant, such as methylphenidate, and adequate hydration by stomach tube will restore their sensitivity to dextran (unpublished).

It was demonstrated previously that susceptibility of rats to anaphylactoid reaction is closely related to the number and integrity of cutaneous mast cells(8). Tissue mast cells have been found unchanged in reserpinized animals(9,10), while 5-HIAA increases in the urine. This picture is the reverse of that seen during anaphylactoid reaction in rats where mast cells are degranulated but there is no concomitant increase of urinary 5-HIAA.

Summary. Urinary excretion of 5-hydroxyindolacetic acid (5-HIAA) has been evaluated in rats treated with various histamine liberators. Excretion of 5-HIAA was

unchanged, even when the liberator was administered repeatedly. When rats were given Compound 48/80 in increasing dosage during 8 days, they were entirely resistant to edema normally produced by other liberators. These animals, however, excreted as much 5-HIAA after injection of reserpine as did the untreated controls. These observations do not militate in favor of a serotonin mediation in development of anaphylactoid edema in rats.

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Metabolic Effects of *m*-Dinitrobenzene in *Aspergillus niger*.* (26184)

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m-Dinitrobenzene (DNB) is a potent growth inhibitor for molds; concentrations as low as 2.5 x 10^{-5} M produce complete stasis of *Aspergillus niger* growth. Casein hydrolyzates and amino acid mixtures reversed inhibition but it was not possible to establish mechanism of action for these phenomena(1). The present communication reports metabolic observations which are consistent with the proposal that DNB inhibits mold growth by indirectly depressing amino acid synthesis during early growth phase.

Methods and results. Culturing procedures for *A. niger* (Mulder strain)(2) and growth rate methods were as described previously (1). DNB solutions were prepared fresh weekly since activity is gradually lost on standing in stoppered glass containers for longer periods. Although growth inhibitory properties of DNB were overcome by various amino acid mixtures(1), leucine and proline were the only amino acids which, when omitted individually from such mixtures, prevented this reversal. These 2 compounds were, in fact, nearly as effective as the whole mixture of 19 amino acids when substituted on an equal weight basis. Hydroxyproline was without effect, but aspartic and glutamic acids were partially effective substitutes for proline. These results are in accord with known metabolic interconversions of amino acids. It is significant that these 4 effective amino acids constitute 50% of the casein molecule.

In addition to amino acids, certain intermediates of carbohydrate metabolism were also effective in reversing DNB growth inhibition (Table I) and these effects were most pronounced between 5th and 6th day of growth. (Decrease in weight of controls is due to autolytic changes after 5th day). An equivalent amount of a mixture of citric, α-ketoglutaric, succinic and oxalacetic acids

TABLE I. Reversal of DNB Growth Inhibition in *A. niger*.*

Additions†	Incubation time (days) 5	6	7
None	836	751	667
1.0 μmole DNB	17	23	59
Idem + leucine + proline‡	194	342	479
" + citric acid	240	811	837
" + succinic acid	39	457	727

* mg dry wt of mycelium (50 ml medium/flask).
† 50 mg of each acid listed.
‡ Added at expense of isonitrogenous amount of NH_4Cl.

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