that immature smooth muscle plays a minor role in the 5-HT neonatal hyposensitivity. There is indirect evidence that the hypothesis of receptor deficit for 5-HT cannot be supported. If receptor deficit were indeed present in the origin of 5-HT hyposensitivity, then a similar decrease in affinity towards amphetamine would be expected. The problem whether 5-HT and amphetamine act on the same receptor is now being investigated.

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Alanosine and hadacidin—Comparison of effects on adenylosuccinate synthetase*

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ALANOSINE [L(–)2-amino-3-nitrosohydroxylamino propionic acid], originally obtained from *Streptomyces alanosinicus* (ATCC No. 15710), has inhibitory activity against a number of viruses *in vitro* and *in vivo*, and induces regression of a transplanted fibrosarcoma.[1] Studies of its mode of action in an ascites tumor system[2] *in vivo* showed that it inhibits the synthesis of RNA adenine from formate-^{14}C and from glycine-^{14}C with no appreciable reduction of the rate of RNA guanine synthesis. The incorporation of preformed adenine-^{14}C into RNA purines is not inhibited. A similar pattern of activity was observed in a microbial system.[3] In addition, the incorporation of aspartate-^{14}C into microbial RNA pyrimidines is inhibited while the incorporation of uridine-^{3}H is enhanced. The fact that inhibition by alanosine of the growth of *Candida albicans* was antagonized by aspartate led to the tentative suggestion that the drug depresses the activity of adenylosuccinate synthetase and aspartate transcarbamylase.

Hadacidin (*N*-formyl hydroxyaminoacetic acid), an antibiotic from *Penicillium frequentans*, has an action mode similar to that of alanosine in that it inhibits the incorporation of formate and glycine into adenylic acid with no inhibition of guanylic acid formation.[4] Subsequent studies of a partially purified adenylosuccinate synthetase revealed the enzyme to be quite sensitive to hadacidin; at an

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aspartate to hadacidin ratio of 2, the reaction velocity was diminished 88 per cent and the inhibition was clearly competitive in respect to aspartate.[5]

Experiments were consequently initiated to determine if the action of alanosine mimics that of hadacidin at the enzymic level.

Alanosine was obtained from Dr. P. Sensi, Lepetit S.*p*.A., Milan, Italy; hadacidin sodium was from Dr. H. T. Shigeura, Merck Sharp & Dohme Research Laboratories, Rahway, N.J., U.S.A.; inosine-5′-monophosphate-8-^{14}C, with a sp. act. of 33 mc/m-mole, was purchased from Schwarz BioResearch, Inc., Orangeburg, N.Y., U.S.A. Other chemicals were of reagent grade from commercial sources.

Adenylosuccinate synthetase (ASase) from *Escherichia coli* B (ATCC No. 11303) was prepared by the method of Lieberman.[6] The final preparation used was his low pH II fraction. The specific activity as determined by Lieberman's u.v. assay method I varied from 7 to 11 in the several preparations. This value was lower than that which he reported, but was of the same order of magnitude found by Shigeura and Gordon in their studies of hadacidin.[5]

For the experiments reported here, it was elected to use a radiochromatographic procedure to detect the conversion of isotopic inosine-5′-phosphate (IMP) to adenylosuccinate (AS). The reaction mixture was virtually identical to that described by Lieberman[6] in his assay I, except that the concentration of carrier IMP was $2{\cdot}1 \times 10^{-5}$ M. After incubation at 37° for 45 min, the reaction was stopped by heating at 80° for 5 min and the precipitated protein was sedimented by centrifugation. Aliquots (100 μl) of the supernatant solutions were spotted onto strips of Whatman No. 1 filter paper and developed (descending) in a solvent composed of isopropanol:HCl:H_2O in volume proportions of 70:16·7:13·3. The developed strips were scanned with a radiochromatographic device (Actigraph III, Nuclear-Chicago Corp.) at 30 cm/hr with a 6 mm collimator slit. Total counts per peak were listed by an attached digital integrator.

The response of ASase to alanosine and to hadacidin is shown in Table 1. The high degree of inhibitory activity of hadacidin was confirmed, and the percentage inhibition obtained with an aspartate to hadacidin ratio of 2 was quite similar to that reported by Shigeura and Gordon.[5] No appreciable inhibition by alanosine could be found up to a concentration of 7×10^{-3} M, at which concentration the aspartate to alanosine ratio was 0·1.

TABLE 1. EFFECTS OF ALANOSINE AND HADACIDIN ON CONVERSION OF INOSINE-5′-MONOPHOSPHATE (IMP) TO ADENYLOSUCCINATE (AS) BY ADENYLOSUCCINATE SYNTHETASE*

Conditions			Per cent conversion of IMP → AS	Per cent of control
Aspartate (M)	Alanosine (M)	Hadacidin (M)		
0	0	0	0	0
7×10^{-4}	0	0	52 (49–57)†	100
0	7×10^{-3}	0	0	0
7×10^{-4}	7×10^{-3}	0	59 (53–65)†	113
7×10^{-4}	10^{-3}	0	54 (49–59)†	104
7×10^{-4}	7×10^{-4}	0	54 (51–59)†	104
7×10^{-4}	10^{-4}	0	50 (46–57)†	96
7×10^{-4}	7×10^{-5}	0	51 (46–55)‡	98
7×10^{-4}	10^{-5}	0	49 (45–55)†	94
7×10^{-4}	0	7×10^{-4}	8 (6–9)†	15
7×10^{-4}	0	$3{\cdot}5 \times 10^{-4}$	14§	25

* In addition to the compounds listed in the "conditions" columns above, each reaction mixture contained, in glycine buffer (0·14 M, pH 8·0): $MgCl_2$, $5{\cdot}7 \times 10^{-3}$ M; adenosine triphosphate, $5{\cdot}7 \times 10^{-5}$ M; phosphoenol pyruvic acid (tricyclohexyl ammonium salt), $5{\cdot}7 \times 10^{-4}$ M; pyruvate kinase, 30 units; guanosine triphosphate, $1{\cdot}4 \times 10^{-5}$ M; inosine-5′-monophosphate, $2{\cdot}1 \times 10^{-5}$ M; inosine-5′-monophosphate-8-^{14}C, 0·25 μc; and enzyme solution, 0·2 ml. The total volume was 0·725 ml. After incubation at 37° for 45 min, the tubes were immersed in water at 80° for 5 min. Radiochromatographic analysis followed as described in the text.

† range of values obtained in 3 experiments.

‡ range of values obtained in 2 experiments.

§ single experiment.

Reproductions of the radiochromatogram tracings obtained from reaction mixtures with and without alanosine and aspartate are shown in Fig. 1. With neither compound present, the only radioactivity found was present solely as IMP (R_f = 0·25) and excluded the possibility of aspartate being present as a contaminant of the glycine buffer (Fig. 1(a)). In the presence of aspartate, over 50 per cent

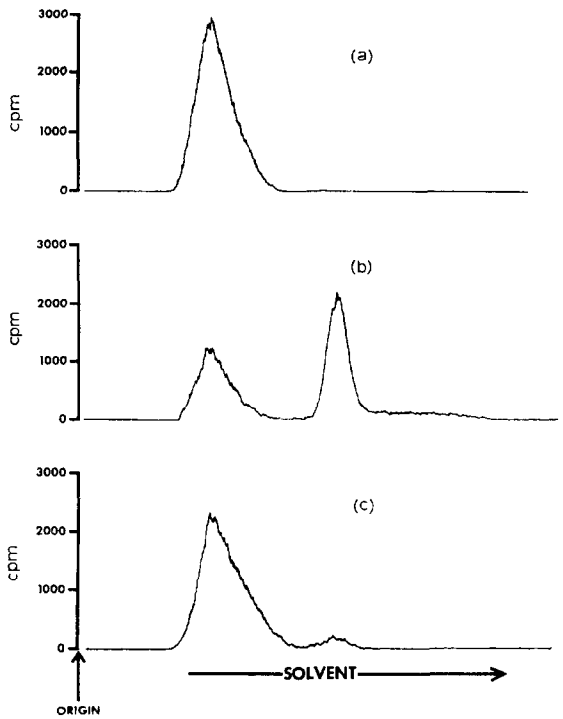

FIG. 1. Radiochromatogram tracings obtained from adenylosuccinate synthetase reaction mixtures with: (a) no aspartate or alanosine; (b) aspartate, 7×10^{-4} M, no alanosine; and (c) alanosine, 7×10^{-3} M, no aspartate. Other components of the reaction were as described for Table 1.

of the IMP was converted to AS with an R_f of 0·49 (Fig. 1(b)). When alanosine replaced aspartate, a small peak was consistently found with an R_f value virtually identical to that of AS (Fig. 1(c)) but which, by reference to Fig. 1(a), could not be AS. Analysis of total counts recorded revealed that approximately 5 per cent of the IMP had been converted to this compound, indicating that alanosine can serve as a substrate for ASase.

Lieberman[6] showed that when NH_2OH replaced aspartate in the ASase system, a product was formed which was detected by an increase in optical density at 280 mμ. Substrate requirements for its formation were the same as those for AS synthesis. Characterization of the product led to its tentative identification as 6-*N*-hydroxy-adenosine-5′-monophosphate. Such a precedent would appear to establish the possibility that the compound formed in the above experiments, when alanosine replaced aspartate, may be 6-*N*-(3-nitrosohydroxylamino propionyl)-adenosine-5′-monophosphate.

In view of the data in the present report, at least two possible explanations exist for the inhibition of RNA adenine synthesis and the absence of notable effect on RNA guanine synthesis demonstrated in intact cells.[2,3] First, the alanosine molecule may be devoid of pharmacological activity until biotransformed in some manner by intact cells to an active compound. This appears unlikely in view of the presence of the highly reactive nitroso and hydroxylamino groups; hadacidin possesses an aldehyde and a hydroxylamino group and is quite active as such. Second, the product formed from IMP and alanosine may be a potent inhibitor of adenylosuccinate lyase in the conversion of AS to

adenosine-5′-monophosphate (AMP). The fact that this latter enzyme also is involved in purine synthesis in the formation of IMP from 5′-phosphoribosyl-5-formamidoimidazole-4-carboxamide should not necessarily predicate against such a hypothesis, since synthesis of the postulated intermediate (lethal synthesis?) at the immediate proximity of the enzymic site may predispose toward preferential inhibition of the enzyme in its conversion of AS to AMP.

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Mechanism of inhibition of histidine decarboxylase (*Clostridium welchii*) by 4-bromo-3-hydroxybenzyloxamine and amino-oxyacetic acid

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SINCE kinetic studies with fetal rat histidine decarboxylase and 4-bromo-3-hydroxybenzyloxyamine (NSD-1055) yielded uninterpretable data,[1] it was decided to ascertain if the bacterial enzyme (*Clostridium welchii*) would lend itself more favorably to such studies. This enzyme was found to be very useful in this respect and the results of these studies comprise the subject of this communication. Also included herein are kinetic studies utilizing amino-oxyacetic acid (AOAA) and 2-hydrazinopyridine (2-HP) as enzyme inhibitors. Since NSD-1055 and AOAA contain the amino-oxy moiety, it was of interest to determine whether the mechanism of inhibition was the same for the two compounds. The third inhibitor, 2-HP, was included for comparison because it lacked the amino-oxy group.

Results obtained with the bacterial enzyme were supported by those obtained with an assay of a specific histidine decarboxylase isolated from rat peritoneal mast cells.

An acetone powder of specific histidine decarboxylase was obtained from Worthington Biochemicals, Inc., and assayed manometrically according to the method of Gale.[2] Specificity of this enzyme for L-histidine di-HCl (Calbiochem) was verified, since 5-hydroxytryptophan (5-HTP) (General Biochemicals, Inc.) and 3,4-dihydroxyphenylalanine (Calbiochem) failed to serve as substrates. An atmosphere of 100% nitrogen was found to improve assay conditions. Addition of pyridoxal phosphate was not required due to tight binding of the coenzyme by the bacterial enzyme.

Histidine decarboxylase was isolated from rat peritoneal mast cells according to published methods[3,4] with certain modifications. Female Sprague–Dawley (Dublin) rats (120–150 g) were injected with 10 ml of heparinized saline. After stunning the animals with a blow on the head, the abdomen was massaged for 2 min to dislodge cells, then opened and the cell suspension removed. Suspensions from all rats were pooled and then centrifuged in 6 ml aliquots at 500 rpm for 3·5 min. The lowest 0·1 ml was resuspended in 1·0 ml of cold, isotonic phosphate buffer, pH 7·4, with 0·2% sucrose. The supernatant was recentrifuged and the lowest 0·1 ml resuspended in 1·0 ml phosphate buffer. Cell suspensions were