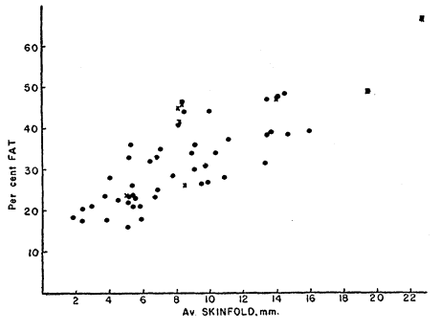

Fig. 1. Percentage of fat in body versus average skin-fold thickness. Females (x), males (•).

tomultipliers goes to the spectrum analyzer dropping into any one of 100 channels depending on the pulse amplitude. Potassium-40 is measured by counting the pulses in the photopeak for a measurement time of 30 minutes. Background interference is reduced by placing the subject and the crystal detector in a room with 8-inch thick steel walls. The system has been calibrated by the measurement of laboratory personnel of a selected size range who ingested known amounts of potassium-42. The sensitivity and reproducibility are such that remeasurement of the same individual gives the same result with a standard deviation of about 1.5 percent. Stable potassium content can be readily calculated from the normal abundance of K^{40}.

Fat content was calculated as the difference between total weight and lean body weight (LBW), on the basis that the latter has a potassium content of 68.1 meq/kg:

$$\text{LBW (kg)} = \frac{\text{measured total K (meq)}}{68.1}$$

This equation is analogous to that in common use for determination of LBW by deuterium or tritium dilution: namely, LBW = total body water/720, where the denominator is the water content per kilogram of LBW.

The range of potassium content for our subjects was 35–58 meq/kg in the males, and 23–52 meq/kg in the females. These values compare favorably with those reported by Anderson and Langham (*10*) in a much larger group of subjects studied at Los Alamos. Calculated fat content was 16 to 48 percent of total body weight in males and 24 to 67 percent in females. These figures support our clinical impression that some of the subjects were obese.

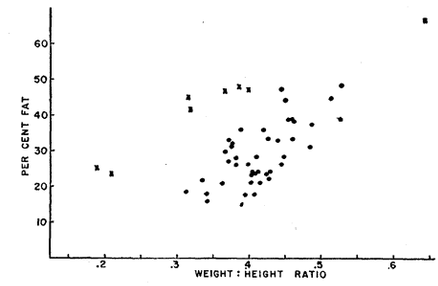

Fig. 2. Percentage of fat versus weight/height ratio (kg/cm). Females (x), males (•).

The relationships between fat content as determined by K^{40} measurement and two other parameters of fatness-leanness are depicted in Figs. 1 and 2. The correlation coefficient of fat content against average skin-fold thickness is 0.80 for the males, and that of fat content against weight/height ratio is 0.56 (males only). The data on females are too few to justify calculation of correlation coefficients for this group. However, the graphs suggest that the trends are similar to those of the males. It is of interest that females tend to have a higher fat content for a given weight/height ratio than do the males.

This report is presented as a new approach to the estimation of fat content in living man. The procedure has the obvious advantage of being nontraumatic and devoid of hazard to the subject. Work is now in progress in a further attempt to assess the validity and accuracy of this method (*11*).

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29 September 1960

Effect of Deuterium Substitution in Sympathomimetic Amines on Adrenergic Responses

Abstract. It was discovered that replacement of the α-hydrogens of tyramine and tryptamine by deuterium produces a marked intensification of the blood pressure effects and nictitating membrane contraction normally produced by these amines. The results are interpreted on the basis of kinetic isotope effects at the level of monoamine oxidase and clearly establish the importance of this enzyme in the limitation of responses when tyramine and tryptamine are involved. The observed deuterium isotope effects with α,α-bisdeuterotyramine (α,α-D_2-tyramine) have been reproduced with only one of the optical isomers of monodeuterotyramine. This establishes that the enzyme displays a high degree of optical specificity. The use of *l*-bisdeuteronorepinephrine revealed that norepinephrine cannot be attacked by the enzyme at the effector cell level.

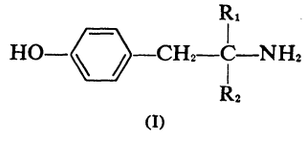
The role of monoamine oxidase (MO) in relation to adrenergic mechanisms has long been a matter of some controversy (*1*). It was suggested some time ago (*2*) that the excitatory properties of drugs such as amphetamine or ephedrine were at least partly related to their inhibitory properties towards monoamine oxidase. In more recent years, the discovery of potent new inhibitors has made it possible to establish an important role for this enzyme in adrenergic mechanisms (*3*). Thus, the administration of iproniazid could be shown (*4*) to potentiate the action of various adrenergic amines, such as tyramine, on the nictitating membrane of the cat. However, no potentiation of the action of the normal transmitter norepinephrine could be demonstrated (*4*). This would seem to indicate that termination of the action of norepinephrine and epinephrine does not involve breakdown through attack by monoamine oxidase. It has been shown by Axelrod and his collaborators that O-methylation is in all probability the mechanism responsible for inactivation of catecholamines (*5*) although, according to Brodie and his group, monoamine oxidase may be involved in the disposition of catecholamines in brain (*6*). However, the demonstration by Carlson (*7*) of the presence of appreciable concentrations of dopamine in brain (as well as serotonin) provides a possibility that the central effects of iproniazid could be related in some way to increased levels of dopamine and serotonin (*6*), both of which can act as substrates for monoamine oxidase.

It has not yet been possible to assess quantitatively and unambiguously the role of monoamine oxidase in adrenergic mechanisms because of the use of large doses of noncompetitive inhibitors

which create conditions that are far from physiological. Moreover, these drugs lack specificity (*8*) (analgesia, hypoglycemia, hypotension, and so forth), and it is likely that in their presence the physiology of adrenergic effector cells is altered (*1*), thus making the interpretation of results difficult.

We have discovered a new approach, which is free of ambiguities, to the problem of the physiological role of monoamine oxidase in adrenergic mechanisms. We wish to report at this time some of the most significant preliminary results which best illustrate the principles involved in this approach.

It was reasoned that, in order to evaluate monoamine oxidase activity in effector cells and other tissues, it would be essential to compare two adrenergic amines stereochemically indistinguishable by monoamine oxidase but differing in susceptibility to degradation by it. Such requirements are not met by the usual series of analogs, isologs, or isosteres because of distinct stereochemical differences between any two compounds. However, the substitution of deuterium for hydrogen atoms in a molecule does fulfill the above requirements, and differences between the rate of degradation of a substrate and its deuterium-labeled counterpart are to be expected if the rate-limiting step of the degradation involves breaking of a carbon-hydrogen bond. As an example, should the adrenergic amine tyramine (which is a good substrate for monoamine oxidase) be labeled with deuterium on the α-carbon (I, $R_1 = R_2 = D$), the rate of its oxidation by the enzyme should be decreased as long as the rate-determining step involves breaking of a carbon-deuterium bond.



(I)

Now, if monoamine oxidase is involved as a limiting factor in the response of a nictitating membrane, the substitution of deuterium for hydrogen in tyramine should lead to quantitative differences in the pattern of membrane contraction. Since the labeled tyramine is stereochemically identical to normal tyramine, any difference in response can be ascribed to variations in binding constants and rate of degradation of the substrate under perfectly controlled physiological conditions. Similar reasoning also applies to norepinephrine which in a similarly labeled form might lead to valuable information about the nature of receptors.

Results. Pentobarbitalized cats were prepared for recording blood pressure and nictitating membrane contractions in the conventional manner. Tyramine and α,α-bisdeuterotyramine (98 percent isotopic purity) (I, $R_1 = R_2 = D$) were administered intravenously in equivalent doses. Figure 1 illustrates the effects of the two materials on arterial pressure and membrane contraction in typical experiments. Table 1 summarizes the results of all studies carried out. The magnitude of the pressor response was essentially the same for both compounds, but the duration of the effect (reflected in pressor area) was twice as prolonged with α,α-D_2-tyramine (statistical analysis giving a p value < 0.001). Both the magnitude and duration of the nictitating membrane contractions were twice as large after administration of α,α-D_2-tyramine as with tyramine ($p < 0.001$). The greater magnitude of contraction observed in the latter instance is probably related to the lack of compensatory mechanisms such as exists for blood pressure maintenance. When α,α-bisdeuterotryptamine was compared with tryptamine under the same conditions, an identical pattern of differences was observed. However, when *l*-bis-α-deuteronorepinephrine (synthesis to be described elsewhere) was compared with *l*-norepinephrine, no difference whatsoever could be observed in their potencies [see Fig. 1 (*1A, 1B*)].

Table 1. Comparison of tyramine and α,α-bisdeuterotyramine on sympathetic receptors in the cat.

	No. tests	Intravenous dose (μg/kg)	Mean maximum blood pressure increase (mm-Hg)	Mean pressor area*	Mean nictitating membrane contraction	
					Maximum (mm)	Area$_{50}$*†
Tyramine	17	800	82	142	11	54
α,α-D_2-tyramine	9	800	84	291	22	179
Ratio: α,α D_2-tyramine / tyramine				2.1	2.0	3.3
p			0.75	<0.001	<0.001	<0.001

* Areas determined with planimeter † Areas under curve to 50-percent recovery.

Stereospecificity of the deuterium isotope effects. In view of the above results, it was of considerable interest to examine the possibility that the observed isotope effects may be stereospecific, that is, assuming a three-point contact between substrate and enzyme or receptor, only one of the two possible

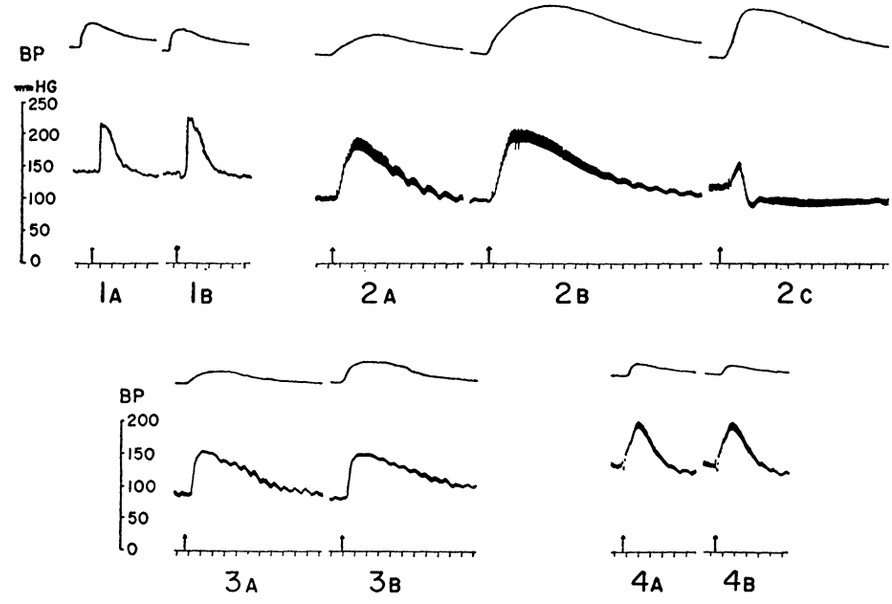


Fig. 1. Effects of tyramine and α,α-bisdeuterotyramine on arterial pressure and membrane contraction. Female cats (3.0 to 3.5 kg), under pentobarbital anesthesia, were used. From top to bottom: isotonic contraction of nictitating membrane, arterial blood pressure, time in minutes. Test compound given at arrows (intravenously): norepinephrine, 16 μg/kg, all tyramine compounds, 800 μg/kg. *1.* Cat 3.1 kg: *A*, *l*-norepinephrine; *B*, *l*-α-bisdeuteronorepinephrine. *2.* Cat 3.0 kg: *A*, tyramine; *B*, α,α-bisdeuterotyramine; *C*, α-monodeuterotyramine (prepared enzymically). *3.* Cat 3.5 kg: *A*, tyramine; *B*, α-monodeuterotyramine (same isomer as in *2C* but prepared synthetically). *4.* Cat 3.2 kg: *A*, tyramine; *B*, α-monodeuterotyramine (opposite isomer of that shown in *2C* and *3B*, prepared enzymically).

optical isomers of α-monodeuterotyramine (I, R_1 = H; R_2 = D; and I, R_1 = D; R_2 = H) may be responsible for the isotope effect observed with the bisdeutero amine. Both optical isomers of α-deuterotyramine were prepared enzymically from tyrosine (*9*) and assayed as above. The results are shown in Fig. 1 (*2C* and *4B*) (*10*) and clearly establish that the isotope effect is completely stereospecific in accordance with a three-point contact between tyramine and monoamine oxidase. The absolute dependence on configuration of the isotope effect on the nictitating membrane response fully agrees with our deductions based on in vitro studies with liver monoamine oxidase (*9*).

Conclusions. From these observations, the following conclusions emerge: (i) The enzyme monoamine oxidase must be intimately associated with adrenergic effector cells and must be an important factor in the limitation of the action of tyramine and tryptamine. (ii) The monoamine oxidase involved in adrenergic mechanisms displays an absolute stereospecificity which is identical to that of liver monoamine oxidase, thus making it probable that these two enzymes are very similar in properties and mechanism of action. (iii) Norepinephrine is not a substrate for the enzyme at the adrenergic effector cell level. This excludes an oxidative deamination of transmitter as part of the sequence of events leading to a response or to inactivation of the substrate. (iv) The role of the enzyme in adrenergic mechanisms can best be pictured as a protective device for the rapid disposition of circulating or endogenous nontransmitter material.

So far as we are aware, this is the first report on the use of kinetic isotope effects in the field of pharmacology (*11*) and constitutes a novel approach that should prove a powerful tool in mechanism studies at the receptor level. A full description of this and related work in progress will be published elsewhere (*12*).

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10. The unusual blood pressure effect of the enzymically prepared tyramine (Fig. 1, 2C) has been traced to the presence of a minute amount of a phenolic impurity which is formed when oxygen is not excluded from the incubation mixture. A purely synthetic sample of optically active α-D-tyramine produced a typical blood pressure response (Fig. 1, 3B).
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5 August 1960

Effects of Amino Acid Feedings in Schizophrenic Patients Treated with Iproniazid

Abstract. Large oral doses of individual amino acids were given three or four times daily for periods of 1 week to schizophrenic patients, some of whom were maintained on iproniazid. Marked alterations in behavior in some patients were associated with the administration of *l*-methionine and of *l*-tryptophan.

Recent reports have indicated that certain amino acids or their endogenous derivatives may affect mental state and behavior, and have led to hypotheses of their involvement in the pathogenesis of some forms of schizophrenia (*1*). Evidence for such hypotheses has been sought in the psychotomimetic properties of certain amines or their congeners (*2*), and in qualitative or quantitative abnormalities among the amino acid products excreted in the urine of schizophrenic patients (*3*).

This report describes a study of the effects of large quantities of certain amino acids given to a group of 12 chronic schizophrenic patients, previously described (*4*), nine of whom were also given iproniazid throughout the study in an effort to increase tissue concentrations of endogenous amines. None had received somatic therapies within the previous 18 months.

The study is summarized in Table 1. There were three time blocks (periods A, B, and D) during which, in a Latin square design, every patient received in rotation each of the tabulated amino acids (or amino acid combinations) for approximately 1 week. During periods C, E, and F the Latin square design was not used. Instead, certain individuals received only one of the substances tabulated, selection depending primarily on their having previously responded to the same, or a related substance. Except as noted in Table 1, the *l* forms of amino acids were given, suspended in chocolate milk, three times daily. The appearance, consistency, and taste of the suspensions were made as similar as possible by adding barium sulfate or flavoring agents as needed.

Behavioral observations were made continuously by nursing personnel. Each patient was evaluated daily by one or more of three psychiatrists. Three additional physicians together examined the patients at weekly intervals and were the only participants aware of the drug and amino acid regimens. All patients were observed for signs of hepatic disturbance, and serum transaminase was measured each week. No hepatic dysfunction was detected. Electroencephalographic, psychometric, and biochemical studies, which complemented the behavioral and psychiatric evaluations, are in preparation.

Marked behavioral changes occurred with *l*-methionine loading in four of the nine patients receiving iproniazid—in two patients on each of three trials, in one on two of three trials, and in one on one of two trials. The major clinical features in these patients were an increasing flood of associations often reaching "word salad," increasing anxiety approaching or reaching panic, increasing tension and motor activity, depression accompanying a brief period of sharply increased insight, an upsurge of hallucinatory activity, and brief intermittent periods of disorientation at the height of agitation.

One such patient, essentially mute for many years, manifested a flood of speech and ideas that was uncontrollable for hours. Another patient, paranoid but usually coherent and in good contact, described a flood of ideas, had a sudden depressed insight into the effects of his psychosis on himself and those about him, was flooded with associations, reached the stage of "word salad," and finally repeated isolated letters continuously.

Most changes disappeared abruptly upon withdrawal of methionine, but concurrently each marked reactor was thought to show some unexpected clinical improvement which persisted for weeks or months. The dose level of iproniazid did not seem to influence the intensity of methionine effects.

The changes cannot be attributed to the metabolic acidosis or persistent gas-