Tissue Distribution of C^{14}-labeled Beta-phenethylbiguanide

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Since the original report by Ungar et al.[1] on the hypoglycemic action of phenethylbiguanide, now more commonly known as DBI, the in vitro effects of this drug have been extensively studied. Under certain experimental conditions it can be demonstrated that the drug will inhibit in a variety of tissues the oxidation of glucose as well as other metabolic fuels.[2,3] At the same time tissue anoxia is produced presumably by the drug's action on the electron transport system.

Some of the in vitro effects of this drug have been suggested to explain its action in vivo; however, in our opinion, these studies have failed to yield any clear-cut information as to how this drug lowers the blood sugar in diabetic animals. A summary of the experimental observations has been reviewed recently by Williams and Steiner.[4]

In an attempt to shed some light on its hypoglycemic mechanism, we have labeled phenethylbiguanide with C^{14} and have conducted a time study of the urinary and body tissue distribution of the C^{14} label.

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The papers are arranged in the order of their presentation and the panel discussions or a summary of such panel discussions follows each group of related papers.

DBI is the U. S. Vitamin & Pharmaceutical Corporation brand name for beta-phenethylbiguanide hydrochloride. The compound also is known as beta-phenethylformamidinyliminourea hydrochloride, and as phenformin. These terms are used synonymously throughout the issue.

EXPERIMENTAL

Synthesis of radioactive phenethylbiguanide†-benzyl cyanide-C^{14}: Benzyl cyanide-C^{14} was prepared by the reaction of an excess of benzyl chloride with sodium cyanide-C^{14} (4.5 gm.) according to the procedure of Adams and Thal.[5]

β phenethylamine-C^{14} hydrochloride: Benzyl cyanide-C^{14} (5 gm.) was dissolved in dry ether (100 ml.) and added dropwise, with stirring, to a slurry of lithium aluminum hydride and dry ether (5 gm. $LiAlH_4$ in 300 ml. of dry ether). After addition of the benzyl cyanide (forty-five minutes) the mixture was refluxed for a further two hours. The excess lithium aluminum hydride was then destroyed by the cautious addition of water and the mixture acidified with 10 N sulfuric acid. The acidified aqueous solution was thoroughly mixed with the remaining ether layer. The ether layer was then separated and discarded, and the aqueous layer twice extracted with 100 ml. portions of ether.

The extracted aqueous phase was made alkaline with dilute sodium hydroxide and extracted four times with 200 ml. portions of ether. This ether extract was dried over anhydrous sodium sulfate and reduced by flash evaporation to a 25-ml. volume. A 25-ml. volume of absolute alcohol was added and the phenethylamine precipitated as the hydrochloride through saturation of the solution with dry hydrogen chloride.

Phenethylbiguanide-C^{14}: β phenethylamine-C^{14} hydrochloride (3.5 gm.) and cyanoguanidine (1.7 gm.) were added to 1 ml. of water and the mixture heated at reflux for three hours. The solution was cooled to 110° C. and 8.7 ml. of 99 per cent isopropanol added with stirring. Agitation was continued for one hour and

† We are indebted to Dr. Seymour L. Shapiro of U. S. Vitamin & Pharmaceutical Corporation for his advice in this synthesis.

then the reaction mixture was set aside to crystallize overnight. The resulting solid was filtered and dried. M.P. 173-175° C. The crude product was recrystallized from methanol. M.P. 176-178° C.

Animal experiments: Twenty-four hour fasted male Sprague-Dawley rats were given phenethylbiguanide-C^{14} at the dosage level of 100 mg./kg. In one series of experiments the phenethylbiguanide-C^{14} was given orally and in the other the intraperitoneal route was employed. The animals were placed in metabolism cages to permit collection of the expired air, urine, and feces. One animal from each group was sacrificed at the end of 1, 2, 5, 8, 12, and 24 hours. The liver, kidneys, heart, lung, spleen, gastrointestinal tract with contents, testicles, epididymal adipose tissue, blood, and an aliquot of muscle from the femur were removed and rapidly frozen with dry ice and dehydrated by lyophilization. To permit more uniform planchetting of the tissue for the determining of its C^{14} content (direct counting), the fat and fat-like substances were removed from the dehydrated tissues with acetone and petroleum ether. Thus the C^{14} content was determined separately on the lipid and lipid-free residue. The data presented here for each tissue are the combined counts for these fractions.

TABLE 1

Summary of per cent recovery of radioactivity following oral administration

	Hours after administration					
	1	2	5	8	12	24
Tissue						
Liver	16.2	13.6	2.4	1.9	1.0	1.9
GI tract and contents	71.4	65.6	47.8	33.1	29.3	16.2
Muscle*	2.1	3.6	0.4	0.9	0.0	0.0
All others†	0.9	0.5	0.2	0.1	0.1	0.1
Urine	7.6	16.5	39.2	—	68.9	97.2
Total	98.2	99.8	90.0	—	99.3	115.4

*Muscle value is calculated on the assumption that the muscle represents 50 per cent of total animal weight.
†Combined recovery of count in kidney, heart, lungs, spleen, testicles, and adipose tissue.

TABLE 2

Summary of per cent recovery of radioactivity after intraperitoneal administration

	Hours after administration					
	1	2	5	8	12	24
Tissue						
Liver	15.9	17.6	2.7	2.5	1.4	0.5
GI tract and contents	16.7	18.3	10.7	22.4	22.4	3.9
Muscle*	11.9	8.2	8.0	5.8	4.5	1.2
All others†	5.7	2.1	1.0	0.3	0.2	0.1
Urine	24.8	32.9	68.5	78.4	84.8	(134)‡
Total	75.0	79.1	90.9	109.4	113.3	139.7

*Muscle value is calculated on the assumption that the muscle represents 50 per cent of total animal weight.
†Combined recovery of count in kidney, heart, lungs, spleen, testicles, and adipose tissue.
‡The reason for this high value is not known.

RESULTS

No measurable amount of C^{14} was found in the expired air. The C^{14} label must therefore be quantitatively recovered in the excretion products and the body tissues.

A summary of the C^{14} recovered in the urine and tissues is given in tables 1 and 2. Since the total of the C^{14} recovered in the heart, kidneys, lungs, spleen, testicles, and epididymal adipose tissues was generally less than 5 per cent, the percentage recoveries for these tissues are combined in the tables. It is apparent that the C^{14} label is almost quantitatively excreted in the urine within twenty-four hours in both the orally and intraperitoneally administered animals.

In figures 1 and 2 are data showing the concentration of phenethylbiguanide-C^{14} per ml. of tissue water. The tissue water content was obtained by the loss in weight by dehydration. Two assumptions are made in these calculations. First, it is assumed that the C^{14} label was distributed uniformly in total tissue water, and second, that the C^{14} label represented unmetabolized phenethylbiguanide-C^{14}. Figures 1 and 2 demonstrate that the stomach plus GI tract and liver concentrated the unexcreted label almost to the exclusion of other tissues.

DISCUSSION

The high C^{14} content in the gastrointestinal tract of the intraperitoneally injected animals was of particular interest to us. In separate experiments the stomachs of three one-hour intraperitoneally injected animals were rinsed out with distilled water. In each case it was found that the chromatographically identified radioactive phenethylbiguanide was predominately in the juice rather than bound to the tissue. It thus appears that the drug is trapped by the acidic stomach juices.

Phenethylbiguanide with a pK_a of approximately 11[6] is probably one of the most alkaline drugs in use today. It is not surprising to find high concentrations of it in the stomach juices. This may speak well for the drug since its slow release from this reservoir would tend to prolong its duration of action. However, in view of the concept of Shore, Brodie, and Hogben[7] that passage across the stomach barrier is accomplished only by an unionized molecule, just how the certainly ionized phenethylbiguanide enters the stomach from the blood in the intraperitoneally injected rats will need further investigation.

It is apparent from the data in figures 1 and 2 that the

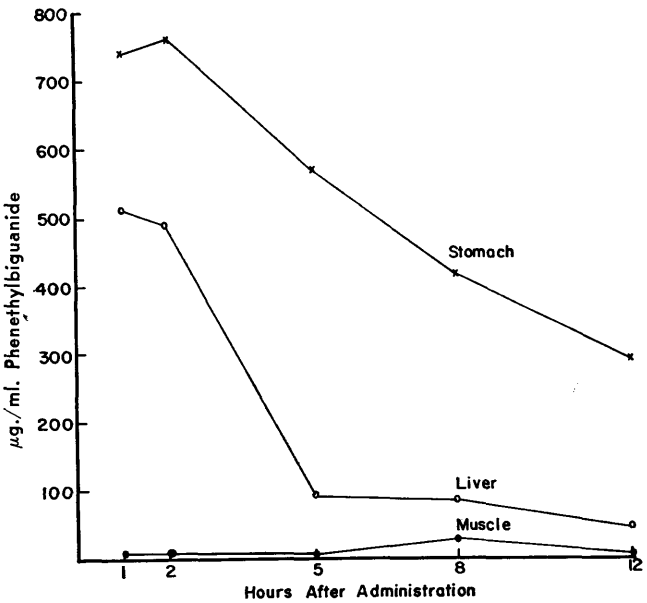

FIG. 1. Phenethylbiguanide (μg./ml.) in tissue water after oral administration. (Stomach refers to gastrointestinal tract and contents.)

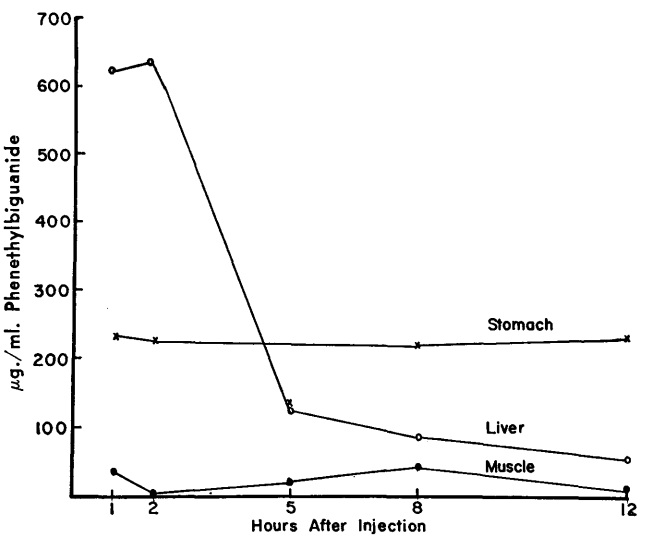

FIG. 2. Phenethylbiguanide (μg./ml.) in tissue water after intraperitoneal injection. (Stomach refers to gastrointestinal tract and contents.)

phenethylbiguanide-C^{14} concentration in the liver at one to two hours was sufficiently high to produce the same metabolic effect as observed under in vitro conditions[2] (i.e., 75 per cent inhibition of the oxidation of C_2 fragments by the Krebs cycle pathway employing adipose tissue). It should be pointed out that as a necessary consequence of following C^{14} distribution, phenethylbiguanide-C^{14} was administered at a dosage level of 100 mg./kg. This quantity is far in excess of that needed to produce a hypoglycemic effect in diabetic animals. It is likewise at least thirty times that normally employed as a human therapeutic dose (assuming a 200-mg. daily dose for a 60-kg. man). However, assuming the distribution of phenethylbiguanide-C^{14} to be the same at 1/30 of the dosage used in this study, sufficient quantities of the drug would still be present to induce the effect observed in in vitro studies at the lower end of phenethylbiguanide concentration gradient (i.e., 25 per cent inhibition of the oxidation of C_2 fragments by the Krebs cycle pathway).

A small inhibitory effect on the Krebs cycle pathway might still produce a major effect in increasing glycolysis in the liver by inhibiting the Pasteur effect. The increased glycolysis would lower sugar reserves and induce a hypoglycemic effect. Excess lactate or pyruvate formed would be conveyed to the muscle tissue to be oxidized by the Krebs cycle pathway there. Indeed the data of these experiments favor our previous suggestion[2] that muscle can carry out the oxidation of excess lactate-pyruvate produced in the liver, since in muscle tissue the phenethylbiguanide-C^{14} concentration was found to be very low.

We realize it is hazardous to draw too extensive a comparison between in vitro and in vivo observations at this time. Greater correlation between in vitro and in vivo effects, if indeed such a correlation exists, must await more detailed observation of the effects of the drug on intact animals, isolated tissues, and purified enzyme systems. Experiments are now in progress to identify the tissue and excretion products of phenethylbiguanide.

SUMMARY

Tissue distribution of the oral hypoglycemic agent, phenethylbiguanide, was determined by oral and intraperitoneal administration of the C^{14}-labeled drug to two separate groups of Sprague-Dawley rats at 100 mg./kg.

It was shown in both groups that the C^{14} label concentrated in the liver and gastric juice immediately following administration of the drug. After five hours approximately 30 per cent of the C^{14} was recovered in the urine and within twenty-four hours over 95 per cent of the C^{14} label was excreted.

SUMMARIO IN INTERLINGUA

Le Distribution Tissutal de Beta-Phenethylbiguanida Marcate con C^{14}

Le distribution in le tissus esseva determinate pro le oral agente hypoglycemic, phenethylbiguanida, post administrationes oral e intraperitoneal del droga marcate

per C^{14} a duo separate gruppos de rattos Sprague-Dawley in un dosage de 100 mg per kg de peso corporee.

Se monstrava in ambe gruppos que le C^{14} esseva concentrate in hepate e succo gastric immediatemente post le administration del droga. Post cinque horas, approximativemente 30 pro cento del C^{14} esseva retrovate in le urina, e intra vinti-quatro horas, plus que 95 pro cento del C^{14} esseva excernite.

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The precise etiology of obesity in man is unknown. The oft-repeated statement that obesity is caused by the ingestion of more calories than are expended in the same time period is factual but is a statement of the conditions which are required for depot lipid deposition rather than a statement of the cause of obesity. This is analogous to saying that the cause of edema is a positive salt and water balance, or that the cause of alcoholism is the excessive ingestion of alcohol. In all three situations, the true causes — and they are undoubtedly multiple — are to be found in the factors causing a disturbance in homeostasis within the organism. In the case of obesity, the problem relates to factors which cause an inappropriately positive caloric balance and eventually an inappropriately high body lipid content. In times of growth, convalescence, pregnancy and lactation, a positive caloric balance is physiologic and essential. In well-nourished adults, a positive caloric balance is unnecessary and undesirable.

The taking of food is, of course, a basic urge which arises within the central nervous system and is required for self-preservation. The homeostatic mechanisms which adjust the intake of food appropriately to the expenditure of energy are not known with certainty and probably involve both neurologic and biochemical pathways and signals. Glucostatic, lipostatic and thermostatic mechanisms have been suggested but none as of this date has been adequately validated in man.

The hypothalamus appears to be the coordinating center for both afferent and efferent impulses which traverse the basic reflex arc adjusting food intake to tissue needs. This reflex may be looked upon as a servo mechanism although the signals which account for both the short and long range control of appetite in relation to exertion and changes in body composition are still unknown. The cerebral cortex undoubtedly exerts an important influence upon this reflex in man. Neural pathways exist from the frontal lobes and other parts of the cortex to the hypothalamus and provide the anatomic basis for the well-established relationship between the psyche and appetite. Certainly psychogenic factors are of great importance in the onset of human obesity. In our own studies we have found that such meaningful and often traumatic experiences as marriage, pregnancy, surgery, menopause, death or separation of a loved one may herald the onset of obesity. Under these conditions "conflicts" arising in the frontal lobes could generate signals which might modify the activity of the satiety center in the ventromedian nucleus of the hypothalamus and lead to relative hyperphagia. Further research is needed to clarify the homeostatic mechanism which operates in the healthy individual so that a more rational basis will become available for pinpointing defects which occur in disease.

From "Obesity as a Nutritional Disorder," by Robert E. Olson, in *Federation Proceedings*, Volume 18, Number 2, Part II, pp. 60-61, July 1959.