Convenient Method for Assay *in Vivo* of Antimetabolites of Serotonin (24046)

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Existing methods for measurement of the potency of antagonists of serotonin employ either isolated segments of smooth muscle or intact animals in which can be observed a more or less specific response to injection of the hormone. Thus, segments of carotid arteries(1) or portions of the uterus taken from an estrogenized rat(2-4) contract when treated with serotonin, and this response can be overcome with suitable antagonists. In this way an *in vitro* assay can be made to measure the potency of such antagonists. *In vivo* measurements can be made by determination of amount of an antagonist which will prevent pressor effects of serotonin injected into a dog(3,5) or to prevent antidiuretic effect of the hormone injected into hydrated rats(6). The *in vitro* assays suffer from the very serious handicap that many antiserotonins which prove to be quite active on the isolated tissues fail to show any significant potency when tested on whole animals(3,5). The *in vivo* assays, such as the one involving measurement of effects on blood pressure, are time-consuming. At least a week of intensive work may be necessary in order to establish the potency of a single compound. Consequently, a more convenient *in vivo* assay may be desirable. The method here described depends on the fact that when a mouse is exposed to a continuous source of serotonin a very severe diarrhea ensues. This can be prevented if the animal has been treated previously with an antimetabolite of the hormone. The continuous source of serotonin is provided by injection of 5-hydroxytryptophane. Udenfriend *et al.*(7) have shown that administration of this amino acid to rats brings about a large increase in serotonin content of most tissues. Because the hydroxytryptophane is converted to serotonin continuously for several hours the increased content of this hormone is maintained in tissues for a relatively long time. This is in contrast to what follows the injection of a single dose of serotonin itself. The difference can be seen readily when one measures increase in blood pressure, or diarrhea. After injection of serotonin an animal frequently defecates because of strong contractions of the intestinal muscles which can be easily observed. The effect is transient however, so that all individuals do not defecate, and those which do, do so only for a very short time. With hydroxytryptophane, however, the contractions continue for a long time. The result is that all individuals exhibit profound diarrhea lasting for a long time. Woolley *et al.*(8) showed that the diarrhea which follows injection of 5-hydroxytryptophane could be completely prevented by prior treatment of animals with a potent antimetabolite of serotonin. This observation has now been made the basis of a method for assay of such antimetabolites in living animals.

Materials. The antimetabolites of serotonin were synthesized in the ways described previously(9-12). The 5-hydroxytryptophane was the racemic form, and was generously supplied by Abbott Laboratories and by the National Drug Co. All compounds were dissolved in Ringer's solution and adjusted to pH 7.5 before injection.

Method. Adult female Swiss mice weighing 25 to 28 g which had been caged for at least one week together in groups of 6 were placed individually in 1 liter beakers for 30 minutes prior to the assay. Any which exhibited diarrhea during this control period were discarded. A group of 6 animals was selected for each dose level of each of the compounds to be examined. Each mouse was then injected intraperitoneally with 0.5 ml of Ringer's solution containing the desired amount of compound to be assayed. Graded doses of each compound were chosen to cover the range of 0 mg up to an amount which would protect

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TABLE I. Diarrhea in Mice Given BAS and 5-Hydroxytryptophane. All mice received 1 mg DL-5-hydroxytryptophane 30 min. after the BAS. BAS = 1-benzyl-2-methyl-5-methoxytryptamine hydrochloride.

BAS, mg/kg	No. of mice	Severe diarrhea	Mild diarrhea
0	6	6	0
4	6	6	0
6	6	5	0
8	6	3	2
10	6	2	1
12	6	2	1
15	6	1	2
20	6	0	0

all 6 mice in the group. A group was also included which received no antimetabolite and no hydroxytryptophane but just Ringer's solution.

Thirty minutes after injection of the antiserotonin each mouse received 1 mg of DL-5-hydroxytryptophane in 0.25 ml of Ringer's solution injected intraperitoneally. The animals were then observed very closely for 30 minutes. The onset of diarrhea, and the severity of it were recorded from visual observation of the excreta in each beaker during this period. It was necessary to keep constant watch of all animals. Mere examination of the beakers at the end of the 30 min. period was not sufficient because if this was done evaporation from watery feces and cleaning of the beakers by the mice tended to obscure the extent of the diarrhea. Severe watery diarrhea of persistent character was seen in all individuals which had received only the hydroxytryptophane. This usually began about 10 min. after injection of the hydroxytryptophane. In those which had been given in addition an amount of an antiserotonin sufficient to confer partial protection, severe diarrhea was seen in some, mild diarrhea in others, and no diarrhea in the remainder. The mild diarrhea might be only the ejection of a few watery droppings from time to time after the passage of some solid feces or it might be one short episode of watery droppings. It was easily distinguished from the severe and prolonged type. The mere passage of normal feces, even though in increased amount, was not counted as diarrhea. Typical responses of mice so treated with one potent antiserotonin are shown in Table I. This compound was 1-benzyl-2-methyl-5-methoxytryptamine hydrochloride, the "benzyl analog of serotonin" or BAS(5).

The quantity of an antiserotonin such as BAS required to protect half of the mice was determined by plotting a dose-response curve from data such as those shown in Table I. For this purpose the number of mice in each group which showed severe diarrhea was plotted against the dosage of antiserotonin. Each mouse with mild diarrhea was counted as 0.5 of a severe-diarrhea-mouse. The amount of BAS required to protect half of the mice did not vary greatly from day to day over a period of 8 months, although there was some variation. Actual values found in 8 experiments were 8.0, 8.5, 9.0, 15.0, 8.0, 12.5, 12.0, and 10.4 mg BAS per kg.

In order to compare the potencies of 2 compounds each was assayed at the same time in the manner described. In the present work BAS was used as standard. The results of such experiments with several compounds are shown in Table II. The amount of an antiserotonin required to protect half of the animals could, of course, be determined more accurately than that needed to protect all, but the usefulness for other purposes of the latter quantity made it advisable to determine and record it as well. It was also found desirable to include in the assay of any antiserotonin dosages ranging from that giving complete protection to that giving none.

The relative potencies of some of the antimetabolites of Table II were known from experiments in dogs in which the ability of each compound to protect against the pressor action of intravenous serotonin had been measured. These relative potencies are included to indicate that although the species and the organ system involved were different, the 2 types of assay rated the compounds in the same order. It is, of course, to be expected that the correlation will not be exact. In fact, it is already known(10) that the route of administration of the anti-metabolites (whether orally or intravenously) can markedly alter the potency in the dog test. However, comparison of results found by intravenous ad-

TABLE II. Amounts of Various Antimetabolites of Serotonin Required to Protect Mice against the Diarrhea Induced by 5-Hydroxytryptophane.

Antimetabolites	Source	Dose for complete protection, mg/kg	Dose for 50% protection, mg/kg	Relative potency in dogs, mg/mg of BAS-phenol
BAS = 1-benzyl-2-methyl-5-methoxytryptamine hydrochloride	(9)	20	10	8
BAS-phenol = 1-benzyl-2-methyl-5-hydroxytryptamine hydrochloride	(10)	8	2	1
BAB = 1-benzyl-2-methyl-5-methoxy-N,N-dimethyltryptamine hydrochloride	(9,11)	32	8	3
3,4,5-Trimethoxyphenoxyethylamine hydrochloride	(12)	40	20	
2,6-Dimethoxyphenoxyethylamine hydrochloride*	(12)	40	22	

* This compound by itself with no hydroxytryptophane caused diarrhea in some mice at 10-20 mg/kg. This may reflect its serotonin-like action on certain isolated tissues(12).

ministration to dogs shown in Table II with those by intraperitoneal administration in mice may indicate that the new method may be of some value in attempts to find useful antiserotonins.

Great precision is not claimed for the present assay method. It might possibly be improved by use of larger numbers of animals in each dosage group, or by other means. The objection which we have found to increase in the numbers is that when more mice are employed the observer's time is so occupied in watching the large numbers that the behavior of individuals is neglected with consequent loss in accuracy. The present procedure seems sufficiently precise to rank antiserotonins in order of activity which is enough for present needs.

Because the dose of 5-hydroxytryptophane used was very large, one might think that the assay could be rendered more sensitive by reduction of this dose. The dose was set so high in order to insure that all of the controls would exhibit diarrhea, and thus simplify the statistical problem which would otherwise arise. Among several hundred mice given the amount of hydroxytryptophane recommended (1 mg) none has failed to show diarrhea. It is true that with the present method rather large amounts of an antiserotonin are needed to protect the animals. This is however of some advantage in attempts to find highly potent antagonists of serotonin because the doubtful cases of compounds with low potency tend to be eliminated. Despite these considerations many experiments were done with smaller doses of hydroxytryptophane and with BAS as the antagonist. These showed that with 0.2 mg of hydroxytryptophane per mouse (still enough to cause diarrhea in all) instead of 1 mg the same quantity of BAS was required as with the larger dose. This fact was probably related to the irreversible character of the antagonism of BAS to serotonin(5). Once a tissue has been protected with this antagonist it will not readily respond even to increased amounts of serotonin. Possibly with reversible antiserotonins a reduction in the challenging dose of hydroxytryptophane might make the assay more sensitive.

The design of the assay made possible experiments on the duration of an antiserotonin effect in an animal. Increases in the time interval between injection of antimetabolite and hydroxytryptophane changed the amount of drug required to give protection of half of the mice, and thus permitted study of this property of persistence of each compound. Because various compounds are excreted (or inactivated) at differing rates, care was taken to observe the same time intervals in all assays, unless persistence was being investigated.

The reason for the control period of time at the start of the assay (the 30 minutes before any compound was injected) was the elimination of animals which will show diarrhea without any antimetabolite or hydroxytrypto-

phane. Unless such mice were eliminated the results were discordant. In order to minimize the number showing such spontaneous diarrhea it was found helpful to cage the animals together in groups of 6 for about a week so that they were not under the stress of being subjected to a new social order immediately before the assay was performed. Because of the possibility of spontaneous diarrhea a control group receiving no drug and no hydroxytryptophane is recommended. This spontaneous diarrhea was not due to salmonellosis or other infections but seemed to occur among a few individuals even though they were in good health.

Summary. An assay for the potency of antimetabolites of serotonin has been described. This assay was rapid and easy to perform and the results were readily reproducible. The recommended method was an *in vivo* one which measured the effects on the intestinal tract. It involved determination of the potency of a compound in causing protection against diarrhea when a large dose of the serotonin-precursor, 5 - hydroxytryptophane, was injected.

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Nucleic Acids and Their Derivatives in Alcoholic Extracts of Rat Tumors and Normal Muscle.* (24047)

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Our object has been to study the chemistry of a highly selected fraction of tumor tissue *i.e.*, an alcoholic extract, because such a tumor extract appeared to have unique biological properties when injected into living tumors, and, as we showed, it also elicited immunological responses of interest in tumor immunology. Earlier studies showed that there were marked differences between substances extracted with alcohol from tumors and from host muscle tissue. For example, in tumor extracts inorganic phosphorus, glucose, lactic acid and ribonucleic acid (RNA) were low, whereas the deoxyribonucleic acid (DNA) was 3-fold high(1). Differences were found also between amino acid composition (2), as well as between protein contents(3) of tumor extracts, and similar extracts made from muscle. The immunology and chemistry of these proteins were studied(3). The present study is limited to investigation of DNA and RNA components which are different in the 2 types of extracts.

Materials and methods. Three sarcomas (Aptekman sarcoma No. 6 and Lewis sarcomas Nos. 7 and 10) originally induced in albino-inbred rats† by injections of methylcholanthrene and then carried on by transplantation, were used. These tumors were of the late generation spindle-cell type following induction by hydrocarbons, described by

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