pituitary growth hormone in biological fluids may be possible.

It was of interest to note in a separate experiment that although antiserum to *bovine* somatotropin was capable of neutralizing the activity of somatotropin from the same species, confirming our previous findings (*3*), it was incapable of altering the activity of *human* somatotropin in the hypophysectomized rat. The species specificity of somatotropin antibodies was thus clearly demonstrated in this instance. A comparative serological study of pituitary somatotropin from various species is in progress (*8*).

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Tranquilizing and Antiserotonin Activity of Nicotinamide

Abstract. Nicotinamide in large amounts antagonized the action of serotonin on smooth muscles in vitro and in vivo. It also tranquilized animals. The antagonism differed in some respects from that of an antimetabolite of serotonin.

Two recent events have suggested that nicotinamide might act as an antagonist to the hormone serotonin. These are (i) the suggestion by Woolley and Shaw (*1*) that the cerebral serotonin content plays a role in mental disorders such as schizophrenia, and (ii) the claim of Hoffer *et al.* (*2*) that massive doses of nicotinamide or nicotinic acid control most cases of schizophrenia.

Woolley and Shaw pointed out that a variety of chemical compounds which they had shown to be antimetabolites of serotonin were able to call forth in normal men and animals some of the signs characteristic of mental disturbances and suggested that this was to be interpreted to mean that the naturally occurring mental disorders of similar type probably arose from an abnormality in serotonin in the brain. Some evidence was found to indicate that the difficulty was a deficiency of cerebral serotonin, but other findings suggested that instead an excess of this hormone might be involved (*3, 4*). It has not yet been possible to decide between these two alternatives. When Hoffer *et al.* announced that large amounts of nicotinamide controlled the disease, the idea occurred to us that if nicotinamide could be shown to act as an antagonist to serotonin this fact would argue in favor of the "too much" alternative.

In isolated smooth muscles and also in whole animals nicotinamide has now been found to act as an antagonist to serotonin. The isolated uterus from estrogenized rats is known to be a suitable test object with which to demonstrate antiserotonin activity (*5*). The muscle was standardized with serotonin, as previously described (*6*). Nicotinamide was applied and the response to serotonin was again measured. Usually nicotinamide caused half maximal inhibition of the contraction when it was present in a final concentration of 0.002*M*, and complete inhibition at 0.01*M* (eight experiments). An occasional tissue required more, but the most needed was 0.01*M* for half, and 0.02*M* for complete, inhibition.

These were the amounts needed to counteract an amount of serotonin just sufficient to elicit a maximal contraction in the untreated muscle. When the dose of serotonin was increased, the amount of nicotinamide required to counteract it was also increased, but not proportionately—that is, the antagonism was not strictly competitive. Once a uterus which had been very sensitive to serotonin (for example, maximal contraction to 0.003 μg/ml) had been treated with an inhibitory amount of nicotinamide, washing would not restore the sensitivity. Such a tissue would, however, respond to larger amounts of the hormone (0.02 μg/ml). The effect of the inhibitor then could be washed away readily, so that the washed tissue would again respond to 0.02 μg. There was thus only loss of the original sensitivity to the hormone, but the newly established level of response could be regained after removal of the nicotinamide.

The specificity of nicotinamide as an antagonist to serotonin was examined by determination of whether it also would counteract the action of acetylcholine on the same muscle. An amount of nicotinamide which would inhibit the serotonin response almost totally reduced only slightly the acetylcholine response (see Table 1). However, larger amounts of nicotinamide did prevent acetylcholine-induced contractions. Washing of the tissue restored the ability to contract to acetylcholine or to serotonin, so that no irreversible damage had been done to the muscle. Nicotinamide was thus not a specific antagonist to serotonin, but in the uterus the antiserotonin action was more prominent than the acetylcholine antagonism.

Table 1. Contractions of an isolated rat uterus caused by serotonin or acetylcholine in the presence and absence of nicotinamide.

Serotonin (μg/ml)	Acetylcholine (μg/ml)	Nicotinamide (*M*)	Contractions (cm)
0.01	0	0	5.5
0	0.04	0	4.0
0.01	0	0.003	0.5
0	0.04	0.003	3.0

Sodium nicotinate did not show any antagonism to serotonin in this tissue, even at 0.1*M* concentration. All solutions were of course adjusted to physiological *p*H in order to avoid the inhibition caused by acidity.

The antiserotonin activity of nicotinamide in living animals was shown in mice treated with 5-hydroxytryptophan according to a method recently described for the evaluation of antimetabolites of serotonin (*7*). This is a severe test of a compound, but 43 mg of nicotinamide gave 50 percent protection against 1 mg of hydroxytryptophan. When the test was made slightly less severe by reduction of the hydroxytryptophan to 0.4 mg per mouse, a complete protection of all mice was observed with 25 mg of nicotinamide.

One prominent feature seen in mice given these massive amounts of nicotinamide was marked lethargy or tranquilization. This was more severe than that seen in mice given reserpine. If lethargy is a feature of antiserotonins which reach the brain (*4*) (reserpine, chlorpromazine, certain synthetic antimetabolites of serotonin) then this effect of nicotinamide is noteworthy in understanding the relationship of serotonin to the mind.

One wonders about the mechanism of the antiserotonin action. Nicotinamide is

Table 2. Antagonism of uterine contraction by various antiserotonins.

Serotonin (μg/ml)	$CaCl_2$ (μg/ml)	Nicotinamide (*M*)	BAS* (μg/ml)	Contractions (cm)
0.01	0	0	0	4.1
0	200	0	0	4.3
0.01	0	0.02	0	0
0	200	0.02	0	0.5
0.01	0	0	1	1.0
0	200	0	1	4.5

* 1-benzyl-2,5-dimethyl serotonin hydrochloride.

not a structural analog of serotonin. This, and the lack of competitive antagonism, would argue against its being an antimetabolite of the hormone. Present data indicate that the action is indirect. Thus, nicotinamide was found to inhibit the muscle-contracting action of Ca^{++}, whereas a specific antimetabolite of serotonin, BAS (*8*), did not (see Table 2). If the action of serotonin on muscle and nerve cells is to transport Ca^{++} into them (*6, 9*), then a specific antiserotonin should not interfere with the serotonin-like action of Ca^{++}. Conversely, an agent which did not antagonize directly the basic action of serotonin, but which interfered with some other steps of the contractile process of muscle (for example, the energy-yielding ones), should also interfere with the action of Ca^{++}. This is what nicotinamide was found to do. Such indirect inhibitions might be of DPNase or the transmethylation processes which are known to be sensitive to nicotinamide. Elucidation of the precise mechanism must await further study.

The main purposes of this note (*10*) are to point out that nicotinamide affects the behavior of normal animals and that its effects are not incompatible with its ability to act as an antagonist to serotonin and to acetylcholine.

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Researches on the Mechanism of Reserpine Sedative Action

In recent years experimental evidence has accumulated on the possibility that reserpine acts as a sedative through release of serotonin (*1*). Such a hypothesis has been suggested and supported by Brodie *et al.*, who have shown (i) that release of serotonin takes place only with *Rauwolfia* alkaloids which have sedative action (*2*); (ii) that central antagonists of serotonin, such as lysergic acid diethylamide (LSD) (*3*), are also antagonists of reserpine (*4*); and (iii) that substances which increase brain serotonin, such as iproniazid, can reverse the sedative activity of reserpine (*4, 5*).

Recently Lessin and Parkes (*6*) stressed the importance of hypothermia occurring after administration of reserpine as an explanation of its sedative activity. But serotonin too can decrease body temperature (*7*). This observation may explain the potentiation of barbiturates by serotonin, since the hypnotic effects of barbiturates are increased when hypothermia is induced (*8, 9*). Finally, in Pletscher's experiments, iproniazid antagonized both serotonin metabolism and hypothermia after reserpine (*10*).

These data prompted us to determine whether a relationship between the sedative action of reserpine, serotonin release, and hypothermia is evident under different experimental conditions.

The sedative action of reserpine was evaluated by the potentiation of sleeping-time after administration of pentobarbital (20 mg/kg, intraperitoneally). Brain serotonin was extracted according to the method of Bogdanski *et al.* (*11*) and measured spectrophotometrically at 275 mμ by the method of Udenfriend *et al.* (*12*). Rectal temperature was determined with a resistance thermometer. The experiments were carried out with 200-g female albino rats kept in constant-temperature rooms at 0°, 22°, 29°, or 37°C and injected intraperitoneally with 2.5 mg of reserpine per kilogram 4 hours before the reported determinations. The results are summarized in Table 1.

Table 1. Results. The figures in parentheses show the number of animals in each series. Each brain serotonin value represents an analysis of the pooled brains of two to three animals.

Treatment	Room temperature (°C)	Time (hr)	Rectal temperature (°C ± S.E.)	Brain serotonin (μg/g ± S.E.)	Sleeping-time after pentobarbital ± S.E.*	Animals that went to sleep/ treated animals
Control	0	21	36.6 ± 0.15 (10)	0.93 ± 0.11 (6)		
Reserpine	0	21	34.3 ± 0.26† (10)	0.99 ± 0.024 (10)		
Control	22	21	37.3 ± 0.09 (25)	0.91 ± 0.05 (30)	25′ ± 1′37″	11/22
Reserpine	22	21	32.6 ± 0.3† (15)	0.26 ± 0.02† (8)	53′ ± 2′13″†	20/22
Control	29	4	37.3 ± 0.4 (5)	0.96 ± 0.03 (4)	24′ ± 1′41″	6/8
Reserpine	29	4	34.4 ± 0.3† (5)	0.29 ± 0.037† (4)	63′ ± 1′45″†	7/8
Control	29	21	37.2 ± 0.18 (5)	1.02 ± 0.034 (4)	24′ ± 1′52″	5/8
Reserpine	29	21	34.5 ± 0.19† (5)	0.31 ± 0.035† (4)	54′ ± 3′35″†	7/8
Control	37	4	37.5 ± 0.14 (5)	1.15 ± 0.04 (4)	23′ ± 2′20″	6/8
Reserpine	37	4	37.2 ± 0.13 (5)	0.85 ± 0.35 (4)	28′ ± 1′14″	5/8
Control	37	21	37.1 ± 0.1 (15)	1.25 ± 0.074 (6)	24′ ± 1′04″	8/16
Reserpine	37	21	37.0 ± 0.12 (15)	1.72 ± 0.24† (10)	24′ ± 1′24″	7/16

* The average includes only the number of animals that went to sleep after receiving pentobarbital.
† Significant beyond the 0.01-level of probability.

Our results show that increasing room temperature from 22° to 37°C does not change body temperature and sleeping-time after pentobarbital but does induce a small increase in the content of brain serotonin. After reserpine is injected, sedation is present only if serotonin is released and body temperature decreases (22° and 29°C). At 37°C, body temperature is unchanged and brain serotonin increases after administration of reserpine; under these conditions there is no evidence of sedative activity. At 0°C reserpine significantly decreases body temperature, with no change in brain serotonin. The measurement of sleeping-time after pentobarbital was very difficult to evaluate because the cold provokes large variations between animals. However, when the rats which had been at 0°C were brought to normal room temperature, it was evident that animals treated with reserpine did not show typical sedation and ptosis. But after 1 to 2 hours at normal temperature sedation appears. An experiment, carried out by keeping the animals after reserpine treatment for 4 hours at 0°C and for 4 hours at 22°C, showed in fact a decrease of brain serotonin (0.63 ± 0.08 μg/g).

Thus, at 22°, 29° and 37°C, there is a parallelism between the onset of the sedative activity of reserpine, as indicated by an increase of barbiturate narcosis, serotonin release, and hypothermia. In contrast, at 0°C we obtained hypothermia after reserpine injection, without sedative activity or serotonin release. It is not simply a delayed action, for 8 hours after reserpine treatment at 0°C we observed no decrease in the content of brain serotonin (1.17 ± 0.06 μg/g).

Our results agree with the hypothesis that the sedative action of reserpine takes place only when there is serotonin release. The onset of hypothermia is not always associated with sedation, and it is not correlated with serotonin release. This is true also in experiments in which we kept the animals in ice bath until their rectal temperatures reached 30°C. Under these conditions no decrease of brain serotonin occurs (1.07 ± 0.1 μg/g).

It is interesting to speculate on the