that they act specifically on the acetylcholine receptor, are new supports for the proposed role of acetylcholine in conduction, especially when considered in connection with the huge amount of physical and chemical data accumulated in the last two decades in favor of this concept.

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An Immunological Investigation of Human Pituitary Growth Hormone

Abstract. Growth hormone isolated from human pituitaries has been demonstrated to be a good antigen in the rabbit. With the rabbit antiserum to human somatotropin, it is possible to detect as little as 0.1 μg of the hormone by precipitin test. The antiserum was also capable of neutralizing the growth-promoting activity of human somatotropin.

It is the purpose of this report to present evidence for the antigenicity of human pituitary growth hormone (somatotropin) in rabbits and guinea pigs. Although Cruickshank and Currie have recently produced antisera to pituitary somatotropin from humans (*1*), they were unable to demonstrate by means of precipitin tests the presence of any antibodies that were hormone-specific. Absorption of the somatotropin antiserum with their human ACTH or TSH preparations resulted in the loss of antibodies to somatotropin itself. Heijkensköld and Gemzell (*2*) did not detect any precipitating antibodies to human somatotropin in the sera of rabbits, guinea pigs, and rats that had been injected with the hormone.

The production of a potent antiserum to highly purified bovine pituitary growth hormone has recently been reported (*3*). This antiserum, freed of certain nonspecific antibodies by absorption, was found to be capable of detecting as little as 0.5 to 1 μg of bovine somatotropin by the precipitin ring test, and it gave no cross reaction with comparable and considerably higher doses of all the other known anterior pituitary hormones. Furthermore, it was observed that relatively small amounts of the antiserum could completely neutralize the biological activity of the bovine somatotropin (*3*).

In the present investigation, highly purified human pituitary growth hormone prepared according to a method previously outlined (*4*), was employed for the sensitization of guinea pigs and the immunization of rabbits. Guinea pigs were readily sensitized to the human somatotropin preparation. A dose of 10 μg of the hormone suspended in 0.4 ml of the adjuvant Bayol-Arlacel (*5*) was injected subcutaneously; the same dose was repeated 3 days later. Three weeks after the initial sensitizing dose, the animals were challenged intracardially with varying doses (0.025 to 0.100 mg) of the hormone in 0.5 ml of saline solution. Eight out of 11 of these animals showed typical signs of severe anaphylactic shock and succumbed within 3 to 5 minutes after the injection, whereas no signs of anaphylaxis following similar doses of somatotropin were demonstrable in six control animals that had undergone no prior sensitization.

The antiserum was prepared in the following matter. Young albino female rabbits, weighing approximately 2.6 to 2.8 kg each, were injected with a total of 4 mg of somatotropin suspended in Freund's adjuvant according to the procedure of Cohn (*5*); of this dose, 2 mg was administered subcutaneously and 2 mg intraperitoneally. Two weeks later the rabbits were bled from the marginal ear vein and were injected the following day with 4 mg of somatotropin in the same manner as before. After another interval of 2 weeks, the animals were each injected with 2 mg of the hormone, 1 mg subcutaneously and 1 mg intraperitoneally, in the alum precipitate form (*6*), bringing the total dose of somatotropin per rabbit to 10.0 mg. The rabbits were bled by cardiac puncture 10 days later, and the serum was frozen and stored until use.

Precipitin ring tests (*6*) were performed with the rabbit antiserum to determine the smallest amount of human somatotropin that could be detected. Tubes were set up containing a serial dilution of antigen ranging from 0.5 down to 0.00005 mg in 0.5 ml of saline. One-tenth milliliter of human somatotropin antiserum from which certain nonspecific antibodies had been removed by absorption was carefully layered under the antigen phase. Tests were read after incubation for 1 hour at 30°C. The minimal amount of somatotropin giving a definite positive precipitin test was 0.1 μg of the hormone.

The antiserum was also tested to determine whether or not it was capable of neutralizing the biological activity of the human somatotropin, as determined by bioassay according to the standard 4-day tibia test in hypophysectomized rats (*7*). The experimental animals, all hypophysectomized, were apportioned into three groups. The first group, consisting of six animals, served as controls and received normal rabbit serum only. The five animals in group 2 were injected with normal rabbit serum plus somatotropin, and the six animals in group 3 received antiserum and somatotropin. The daily dose of hormone was 0.010 mg in 0.5 ml of saline solution, injected subcutaneously, and the daily dose of serum was 0.25 ml, administered intraperitoneally. The injections of serum were begun 4 hours prior to the first injection of hormone, so that over the 4-day injection period, five injections of serum were administered. The animals were sacrificed approximately 24 hours after the final injection of hormone. The results showed that the total dose of 0.040 mg of somatotropin had increased the width of the tibial epiphyseal cartilage plate to 237 ± 4 μ (mean ± standard error), as compared with 160 ± 1 μ for the controls that had received normal rabbit serum only. The simultaneous administration of antiserum with the somatotropin had resulted in a complete neutralization of the hormonal effect (164 ± 3 μ). It has previously been demonstrated (*3*) that the injection of bovine somatotropin antiserum alone does not have any significant influence upon the width of the tibial epiphyseal cartilage plate in the hypophysectomized rat (157 ± 4 μ, as compared with 161 ± 3 μ for the uninjected hypophysectomized controls). The fact that the biological activity of somatotropin was neutralized completely when the hormone and relatively small amounts of the antiserum were injected into two entirely distinct sites by two different routes in the same animal suggests the specificity of the antigen-antibody reaction in question. The findings from the precipitin tests, supported by the results of the antihormone tests, have further suggested that an immunological method of assay for the presence of

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.