val (80.0 percent cumulative mortality). Thus, rat macroglobulin is equally as effective as mouse macroglobulin in producing enhanced survival in irradiated BC_3F_1 mice. Horse macroglobulin was only marginally effective.

In recent years, the serum alpha globulins fractionated by various procedures have been investigated. Mowbry and Hargrave (*7*) described an immunosuppressive effect of heterologous alpha globulins in mice injected with rat erythrocytes (RRBC). Repeating the experiments of Mowbry and Hargrave with our macroglobulin fractions (mouse, rat, and horse), we found no effect on the serum hemagglutinin response to RRBC as compared with controls that were not treated with macroglobulin.

The increased concentration of a serum alpha-2 protein, often designated "acute phase macroglobulin," has been described in man, monkey, dog, rabbit, rat, and chicken under various physiological and pathological conditions (*8*). Several hypotheses have been advanced to explain the appearance of this macroglobulin. One hypothesis is that the etiological agent stimulates a synthesis of the protein present ordinarily in trace amounts (*9*). A survey of conditions in which the protein was detected reveals the common factor of tissue injury or cell death, or both. It is interesting to note that 24 hours after *Salmonella* sp. endotoxin injection into rats (*8*) and mice (*10*), an increase in this protein has been detected. Thus, endotoxin is one of the many agents that can stimulate the increased concentration of this alpha-2 macroglobulin. It has also been well-established that endotoxin injected 24 hours prior to irradiation promotes optimum changes of survival (*11*).

Since the active material which we have isolated is associated with the alpha macroglobulin fraction, further investigations are warranted to determine if indeed we are concentrating the trace amounts of "alpha-2 (acute phase) macroglobulin" considered normally present in rat and mouse. Also, this may prove to be a means of determining whether the radiation-survival activity of the endotoxin is mediated through this macroglobulin.

The recovery-enhancing effect of serum macroglobulin in mice shown in these experiments has proved to be highly reproducible. The seven survival studies with isologous macroglobulin were carried out over a period of 1 year from four individual macroglobulin preparations obtained from separate batches of fresh mouse serum. We are confident that the active material is an intrinsic serum constituent associated with the 19*S* protein of mice and rats, and functions across species barriers.

Bioassay for erythropoietin, in polycythemic C_3H mice (*12*), showed that no detectable erythropoietin (> 0.05 units) was present in 3 mg of either the first or second peak of BC_3F_1 mouse-serum protein preparation.

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Serotonin Release from Brain Slices by Electrical Stimulation: Regional Differences and Effect of LSD

Abstract. *Slices of rat brain which had accumulated tritiated serotonin either in vivo or in vitro were superfused and electrically stimulated. There occurred a marked release of the exogenous amine and, to a lesser extent, its deaminated metabolites, which varied with the region of brain tested and was inhibited by lysergic acid diethylamide.*

The status of serotonin as a transmitter substance within the mammalian central nervous system remains uncertain despite its occurrence within the synaptic vesicles of certain nerve terminals (*1*) and the effects of its microelectrophoretic application upon the activity of some neurons (*2*). Although biochemical and histochemical evidence of depletion of serotonin after electrical stimulation of specific neural pathways has been reported (*3*), there has been no direct demonstration of release of serotonin in response to nerve cell stimulation in the brain of the living animal. The difficulties associated with the study of serotonin release in vivo have prompted the development of various techniques in vitro. Anden and co-workers (*4*) reported the enhanced efflux of endogenous serotonin from isolated spinal cord by prolonged electrical excitation. The electrical stimulation of brain slices has been shown to liberate exogenous catecholamines (*5*). We now report on the release of tritiated serotonin under similar conditions.

Brain slices, when incubated in a suitable medium, maintain their bioelectrical and biochemical activity for several hours (*6*). Electrical stimulation of such tissues has been shown to displace resting membrane potentials (*7*). Labeled serotonin is accumulated by brain slices (*8*) and, when administered in low concentrations, appears to be largely concentrated within nerve terminals which normally contain serotonin (*9*). There is also electron-microscopic-autoradiographic and fluorescent histochemical evidence suggesting the uptake of serotonin within the synaptic vesicles of presumably serotonergic nerves when the amine is introduced into the cerebrospinal fluid of the living animal (*10*). Accordingly, we attempted to release exogenous serotonin from slices derived either from animals which had received an intracisternal injection of labeled serotonin or from animals that were incubated with the amine in vitro. Similar results were obtained in both cases.

Tritiated serotonin (20 μc, 2 c/

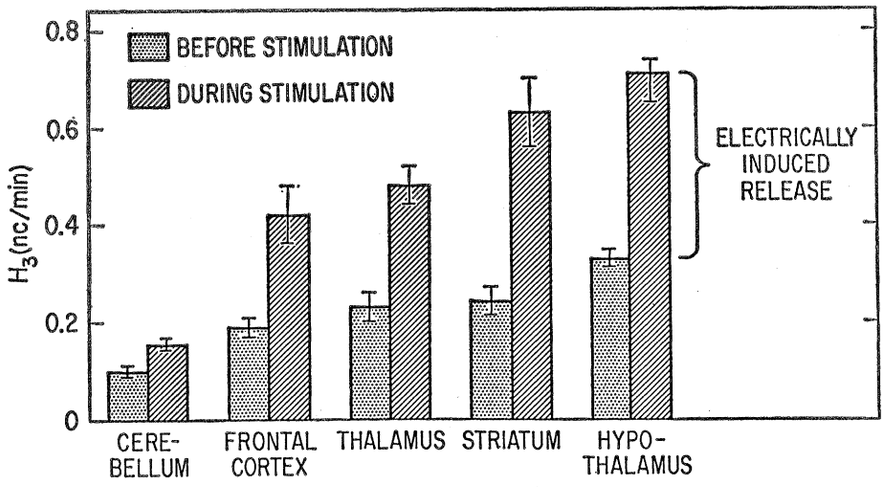

Fig. 1. Efflux of radioactivity from slices from various regions of rat brain after an incubation period of H^3-serotonin. Slices were incubated for 30 minutes with 300 nc of H^3-serotonin per milliliter. Results are the mean values ± standard error of the mean for four or more 20-mg slices before and during stimulation with an 8-volt sinusoidal current at 60 cycle/sec applied for 1 minute.

mmole) was injected into the cisterna magna while the rats (adult Sprague-Dawley males) were lightly anesthetized with ether. The animals were killed 15 to 20 minutes later. In other experiments, slices prepared from the brains of unanesthetized rats were incubated for 30 minutes at 37°C in a supplemented Krebs-Ringer solution (*11*) and saturated with 5 percent carbon dioxide in oxygen, to which H^3-serotonin (300 to 800 nc/ml) had been added.

Slices (20 mg) containing labeled serotonin (either from injection in vivo or incubation in vitro) were held between platinum coil electrodes in individual chambers through which fresh oxygenated medium (37°C) was rapidly circulated. The efflux of radioactivity followed a multiphasic course durir.g 30 minutes of superfusion, similar to that observed with H^3-norepinephrine (*5*). After 20 minutes, about 75 percent of the initially accumulated tritium was lost from the slice. Presumably, washout of the less specifically bound and more rapidly metabolized serotonin and of its metabolites is favored during this period. The 1-minute application of a sinusoidal current 20 minutes after the initiation of superfusion resulted in a rapid, but brief, rise in the efflux of radioactivity. Assay of the superfusate collected just before stimulation disclosed that the serotonin accounted for approximately 35 percent of the total tritium efflux. During stimulation, the proportion of serotonin in the tritium efflux rose to about 60 percent.

While the spontaneous efflux of radioactivity just prior to stimulation increased in proportion to the concentration of H^3-serotonin in the incubation medium (between 6 and 360 ng/ml), no increase in the electrically induced release (rise in tritium efflux during the 2-minute interval which includes stimulation above the level of the preceding 2 minutes) occurred at bath concentrations above 70 ng/ml. The demonstration of stimulus-induced release in these experiments thus depended upon the use of relatively low concentrations of serotonin in the incubation medium. The threshold for detectable release occurred at about 4 volts (15 ma). Release increased in proportion to stimulation amplitude to about 9 volts, above which little or no further increase in release could be demonstrated.

Marked regional differences in the electrically induced release were found (Fig. 1). Release from cerebellar slices was considerably less than that obtained from the other areas examined. Moderate release was obtained from frontal cortex, while striatum and hypothalamus had the largest electrically induced release of any of the areas tested. The liberation of H^3-serotonin from slices of rat liver and salivary gland was quantitatively similar to that obtained from cerebellar tissue. Thus, the amount of release induced by stimulation apparently followed the endogenous serotonin content (*12*) in each of the tissues studied. Nonspecific processes may account for the small amount of electrically induced release obtained in tissues such as cerebellum, liver, and salivary gland, which contain few if any serotonergic terminals. Conversely, a high density of serotonergic terminals appears to be a requisite for the marked release found with diencephalic tissues.

Several tests of the specificity of release observed under the conditions of these experiments were performed. No electrically induced release of either H^3-water or C^{14}-urea could be demonstrated. We were unable to obtain release of H^3-serotonin from slices prepared from brain left in place for more than 8 hours after death or subjected to freezing and thawing after the incubation period but prior to superfusion. No *p*H or significant rise in temperature occurred during the period of electrical stimulation.

Raising the concentration of potassium to 40 mmole per liter in the superfusing medium resulted in a rapid increase in the tritium efflux, which was quantitatively similar to that produced by electrical stimulation, and consistent with the well-established effect of high potassium concentrations to depolarize neuronal membranes (*13*).

The addition of $2 \times 10^{-4}M$ lysergic acid diethylamide (LSD) to the perfusate reduced the electrically induced release of tritium from striatal slices to 63 ± 9 percent ($P < .01$, $N = 10$) that of control slices. The rise in H^3-serotonin produced by stimulation in control slices was 7-fold, but only 2.5-fold in the slices treated with LSD. No consistent effect of LSD at this concentration could be demonstrated in slices derived from the hypothalamus.

Our results, similar to those obtained with norepinephrine (*5*), suggest that this technique may be useful in further studies of the depolarization-induced release of putative central transmitter amines.

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Induced Hypersensitivity to Barbital in the Female Rat

Abstract. *Female rats, treated with two daily anesthetic doses of barbital, exhibit 1 month later a significant increase in sleeping time over that of control animals. Hypersensitive animals, as compared to controls, show no alteration in liver weight (as percentage of body weight), but they manifest a significant shortening of time for induction of anesthesia. Induced hypersensitivity to barbiturates is apparently not the result of alterations in the metabolism of these agents, but it may be related to enhanced susceptibility of the central nervous system to these drugs.*

The induction of delayed hypersensitivity to the depressant effects of pentobarbital on the central nervous system of the rat, after induction of acute tolerance to the drug (*1*), might be related to a decrease in the rate of metabolism of the barbiturate, an alteration in the distribution of the drug in vivo, or enhancement of the sensitivity of central neuronal systems to the agent. We now report on the use of barbital (which is not normally metabolized to any significant extent in the rat) to determine whether hypersensitivity could be developed to this agent. In addition, liver weights were determined, as an index of the activity of hepatic enzyme, and times of induction of anesthesia were recorded on the premise that an increase in central nervous responsiveness to the drug should be reflected in a reduced time of induction of sleep with the drug.

Table 1. Mean sleeping times (± standard error) of female rats receiving sodium barbital (200 mg/kg) intraperitoneally. The figures in parentheses refer to numbers of animals. Group A, animals not previously treated; group B, those treated 24 hours in advance; and group C, those treated 28 and 29 days previously.

Group	Mean sleeping times (min)			
	Day 1	Day 2	Day 29	Day 30
Control	Saline	Saline	Saline	Saline (18)
A	Saline	Saline	Saline	261 ± 15.8 (19)
B	Saline	Saline	221 ± 10.8 (25)	224 ± 12.7 (22)
C	234 ± 12.0 (23)	214 ± 10.2 (21)	Saline	323 ± 14.6 (18)

Four groups of female rats of the Holtzman strain were used. All groups were held in separate quarters for 20 days prior to the initiation of the study to insure acclimation to the laboratory environment. All animals weighed from 134 to 174 g on day 1 of the study and from 176 to 220 g (mean = 196 g) on days 29 and 30. These rats received either 0.9-percent saline (6.67 ml/kg) or sodium barbital (200 mg/kg), prepared as a 3-percent aqueous solution, on days 1, 2, 29, and 30 of the study (Table 1). All injections were intraperitoneal. Induction times and sleeping times for those animals receiving the barbiturate were measured as reported previously (*1*). Any animal with an induction time greater than its group mean plus 4 standard deviations was omitted from the reported data. All error estimates refer to standard error (S.E.).

Table 1 gives the mean sleeping times on day 30 for animals not previously treated (group A), for those treated 24 hours in advance (group B), and for those treated 28 and 29 days previously (group C). Application of the Student *t*-test failed to reveal a significant reduction in sleeping time in group B as compared to group A. This failure to demonstrate significant tolerance to barbital, with a two-dose schedule, is in accord with other reports that stress the difficulty of inducing acute tolerance to barbital in the rat (*2*). The mean sleeping time of group C, however, was 23.8 percent greater than that of group A. This proved to be a statistically significant increase ($P = .01$). These results are similar to those previously obtained with pentobarbital (*1*). Such delayed barbital hypersensitivity is probably not due to alterations in the metabolism of the drug, because, in nontolerant animals, only 3.7 percent of a dose of barbital undergoes biotransformation (*2*). Therefore, even complete inhibition of metabolism could not readily account for the observed increase of sleeping time in hypersensitive animals. Similar conclusions may be made on the basis of observations of liver weight. The mean liver weight (as percentage of body weight) ± S.E. on day 30 for the control group, and groups A, B, and C was 3.70 ± 0.05, 3.63 ± 0.07, 3.89 ± 0.07, and 3.60 ± 0.05, respectively. These estimates are based on the same number of animals as in the day-30 column of Table 1. Only in the case of the tolerant group is liver weight significantly changed from that of the control group ($P = .05$). This increased liver weight indicates that accelerated protein synthesis and enhanced activity of hepatic metabolizing enzyme occurred. This is corroborated by the observation (made by other workers) that acute barbital treatment fails to produce autotolerance, but does cause tolerance to other barbiturates to be manifested (*3*). Also, since the mean liver weight was the same in groups A and C, it is unlikely that depletion of protein precursors, during the phase of enzyme induction, with a resultant decrease in activity of hepatic metabolizing enzyme, could account for a postulated reduction in biotransformation rate, even if the latter could be held responsible for the development of hypersensitivity. This lack of effect on liver weight corroborates similar observations in which significant hypersensitivity to pentobarbital was induced in female rats (*4*).

The mean anesthetic induction time for barbital in 44 rats receiving a first injection of the drug (group B on day 29 and group A on day 30) was found to be 51.0 ± 2.6 minutes. For groups B and C on day 30, the mean induction time was 49.3 ± 4.1 and 38.1 ± 3.5, respectively. The induction time of the rats in group C was significantly shorter than that of both the group receiving a first injection of barbital