Procain bewirkte eher eine Steigerung der Transmitterabgabe, die aber nicht signifikant war. Mit der Beeinflussung der Noradrenalinabgabe ging eine gleichsinnige Beeinflussung der positiv chronotropen Reizwirkung einher (Figur). Doch wurde der Frequenzanstieg *signifikant* erst durch $1,5 \times 10^{-6} M$ Clonidin gehemmt. Die nicht eingezeichnete positiv inotrope Wirkung wurde analog modifiziert. Nach Beendigung der Infusionen und 13minütiger Durchströmung mit reiner Nährlösung lagen Noradrenalinabgabe, Frequenz- und Amplitudenanstieg bei R_3 wieder im Bereich der unbehandelten Kontrollen.

Diskussion. Die Reaktionen des isolierten Kaninchenherzens auf elektrische Stimulierung der postganglionären sympathischen Nerven werden durch $1,5 \times 10^{-6} M$ Procain und Tetracain nicht vermindert, ebensowenig durch $1,5 \times 10^{-5} M$ Procain, während sie durch $1,5 \times 10^{-5} M$ Tetracain fast beseitigt werden. Von Clonidin dagegen, das als Infiltrations-Anästhetikum 1,75 mal so stark wie Procain[1], damit aber erheblich schwächer ist als Tetracain[10], ist nur der tausendste Teil dieser Konzentration erforderlich, $1,5 \times 10^{-8} M$, um die Noradrenalinabgabe bei Sympathikusreizung signifikant zu hemmen. Diese Versuche schliessen nicht aus, dass die peripheren antiadrenergen Wirkungen des Clonidins und des Tetracains auf dem gleichen, nämlich «lokalanästhetischen», Mechanismus beruhen; ebensowenig kann bisher ausgeschlossen werden, dass die Blockade adrenerger Neurone durch Propranolol[11] oder durch Guanethidin, Bretylium und Verwandte[12] im Detailmechanismus lokalanästhetisch ist. Die postganglionären sympathischen Nerven wären dann aber gegenüber Clonidin um mehrere Zehnerpotenzen empfindlicher als gegenüber anderen an sensiblen oder motorischen Nerven gleich oder sogar stärker wirksamen Lokalanästhetika; oder Clonidin hätte einen besonders günstigen Zugang zu sympathischen Fasern. Die Hemmwirkung des Clonidins tritt bei Konzentrationen ein, die Kontraktionskraft und Frequenz nicht verändern; die des Tetracains erst bei einer stark kardiodepressiven Konzentration.

Dass die positiv chronotrope Reizwirkung durch Clonidin verhältnismässig weniger beeinträchtigt wird als die Noradrenalinabgabe, mag dazu beitragen, dass der periphere Angriffspunkt des Clonidins meist übersehen wurde. KOBINGER[2] fand nur eine geringe Abschwächung der durch N. accelerans-Reizung verursachten Frequenzsteigerung bei Katzen und keinen Anhaltspunkt für eine «spezifische adrenerge Neuronenblockade». Neuerdings zeigten aber in Übereinstimmung mit unseren Befunden SCRIABINE et al.[13], dass beim Hund Clonidin die Herzfrequenz durch eine Wirkung auf postganglionäre sympathische Nerven senkt und die Beschleunigung bei Accelerans-Reizung inhibiert. Blockiert wurde auch die elektrisch induzierte Kontraktion und Acetylcholinabgabe des isolierten Meerschweinchen-Ileums[14]. Die Hemmung adrenerger und cholinerger Neurone in niedrigen Konzentrationen rückt das Clonidin in pharmakologische – wie chemische – Nähe zu dem «adrenergisch-cholinergischen Neuronenhemmstoff» 2-(2,6-Dimethylphenylamino)-4H-5,6-dihydro-1,3-thiazin[15,16].

Summary. The influences of clonidine, tetracaine and procaine on the effects of electrical stimulation of the postganglionic sympathetic cardiac nerves have been compared in the isolated perfused rabbit heart. Much lower concentrations of clonidine than of tetracaine were necessary to antagonize the output of noradrenaline and the rise of frequency and contractility. Procaine even in the highest concentration tested did not inhibit the effects of nerve stimulation. In addition to its known central depression of sympathetic tone, clonidine exerts a specific inhibitory action on postganglionic sympathetic neurons.

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The Effects of Intracerebrally Administered RNA, Tubercidin and Histones on Learning in Rats

There is now considerable agreement that extracerebrally administered RNA has no effect on learning in rodents[1,2]. No assessment, however, is available as to the effect and distribution of intracerebrally administered RNA. This was examined along with possible effects of Tubercidin and lysine- and arginine-rich histone fractions on the learning of rats in a shuttle-box avoidance situation.

Materials and methods. Yeast RNA (Pabst) or ^{14}C-RNA (Schwartz) was used as purchased or purified and dialyzed according to the method of CRESTFIELD et al.[3]. Tubercidin (7-deazaadenosine) (TU) was provided by Upjohn Pharmaceutical Company. The lysine- and arginine-rich histone fractions from calf brain cerebellum were prepared, purified, and identified according to JOHNS[4]. Samples of ^{14}C-RNA from tissues[5] were dissolved in NCS reagent (Amersham-Searle) and radioactivity determined by liquid scintillation counting.

Unanesthetized rats received intracerebral (i.c.) injections through small capped cannulae implanted at a depth of 1 mm into the right cortex 3.5 mm from both the sagittal and coronal sutures. The recovery period

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was 2–4 weeks after implantation before commencement of experiments. Injection volumes not exceeding 10 µl were delivered by a Hamilton 10 µl syringe. The intracerebral injections began 17 h prior to the first training period and continued daily throughout training.

150 male albino (Sprague-Dawley) or Long-Evans hooded rats were used. Rats were 125 days old (300 to 350 g) at the onset of testing. All animals were handled daily for 2 weeks prior to the initiation of training.

A series of 8 experiments utilized a 2-chamber wood shuttle-box with copper grid floor. 5 sec after the initiation of the conditioned stimulus (light in 6 and auditory in the final 2) a 0.5 milliampere (ma) shock was passed through the grid on the same side as the conditioned stimulus. An animal was credited with a learned response if it avoided the shock. Latency was also recorded. All experiment were run double-blind, i.e., those involved with injecting or testing the animals were not informed of the substances injected or the grouping of the animals.

Statistical analysis. Results obtained from the animals for response, latency, and acquisition were analyzed statistically by IBM-7044 digital computer with a program for variance analysis (ANOVA Type 1 design)[6].

Results and discussion. Earlier studies[7,8] indicated that the label derived from ^{14}C-RNA administered i.p. or i.v. was not incorporated in the DNA or RNA fractions of the brain. The experiments with ^{14}C-RNA were to test the extent of spread and distribution of i.c. administered RNA, and the difference in the actual radioactivity in cerebral RNA following i.p. and i.c. injections. The time of sacrifice of our animals corresponded to the schedule employed in behavioral testing where 17 h elapsed between injection of RNA and testing. Our studies demonstrated, as tabulated, that radioactivity could be recovered from all parts of the brain following i.c. injection of yeast ^{14}C-RNA although there was some localization in the right cortex around the cannula. Over 80% of the i.c. injected label disappeared in 17 h from brain. The presence of labelled nucleotides in rat brain following i.c. administration of yeast ^{14}C-RNA would support an active cerebral degradation of the exogenous RNA. The amount of unhydrolized yeast RNA remaining 17 h after injection in the rat brain could not be determined by the present methods since there is no analytical way of distinguishing between the yeast and brain RNA in a tissue extract. We also found appearance of small amounts of label in cerebral RNA following i.p. administration of ^{14}C-RNA. In the behavioral testing, there were no statistically significant differences among groups treated with i.p.

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Table 1. Recovery of radioactivity from rat brain RNA following i.p. or i.c. administration of yeast ^{14}C-RNA

	Intraperitoneal		Intracerebral	
	Total DPM	DPM/mg RNA	Total DPM	DPM/mg RNA
Whole brain	15,000 (0.05% total injected)	376 (9.9% brain ^{14}C in RNA)	19,500 (13.9% total injected	4,862 (25% brain ^{14}C in RNA)
Cerebellum	2,398	406	1,872	3,173
Left cortex	3,206	289	2,197	1,979
Right cortex	3,760	339	7,868	7,088
Medulla	1,625	185	1,212	1,377
Subcortical areas[a]	3,559	516	6,300	9,138

Male albino rats sacrificed 17 h after injection. Ip animals received 50 mg ^{14}C-RNA (2.8×10^7 dpm). Ic animals received 250 µg (in 5 µl) (1.4×10^5 dpm). [a] Brain remaining after removal of cerebral cortex, cerebellum, and medulla.

Table II. Effect of i.c. histone and Tubercidin on performance in a shuttle box

Stimulus	No. of animals	Intracerebral injection	Average avoidance resp. ± S.D.	Latency resp. ± S.D.
Visual	10	Lysine-rich histone (100 µg)	7.0 ± 4.3	4.5 ± 1.2
	8	Tubercidin (2.5 µg)	3.6 ± 2.1	5.9 ± 2.6
	8	Saline	4.3 ± 4.3	5.4 ± 1.3
Visual	12	Lysine-rich histone (100 µg)	5.1 ± 3.5	5.1 ± 0.9
	12	Saline	4.3 ± 3.7	5.1 ± 0.9
Auditory	13	Arginine-rich histone (100 µg)	8.8 ± 2.6	4.9 ± 0.9
	13	Albumin	9.4 ± 3.2	3.6 ± 1.1
	13	Saline	9.6 ± 2.2	3.8 ± 0.7
Auditory	12	Lysine-rich histone (100 µg)	7.9 ± 3.5	4.2 ± 1.1
	12	Arginine-rich histone (100 µg)	8.5 ± 3.4	3.9 ± 1.0
	12	Saline	8.4 ± 2.8	4.9 ± 1.0

Male hooded rats. None of the differences are statistically significant.

or i.c. RNA, hydrolyzed RNA or saline with respect to either acquisition or latency.

However, these experiments with yeast RNA would shed no light on the possible involvement of RNA synthesis or DNA derepression on learning as some of the more sophisticated theories imply[9]. Since results of experiments with several of the antibiotic inhibitors of RNA and DNA synthesis are somewhat controversial[9], an attempt was made to influence the learning ability of rats through i.c. injected TU[10]. This antibiotic is an inhibitor of DNA, RNA, and protein synthesis through its incorporation in place of adenosine[11]. In several experiments non-toxic amounts of i.c. TU did not affect the learning of a shuttle-box avoidance situation. An example of this is included in the table of results, along with those obtained with various histone fractions. Lysine-rich histone fraction from calf cerebellum was injected i.c. to assess if such administration would influence, possibly through binding to DNA, the synthesis of m-RNA. There is evidence from in vitro experiments that histones are inhibitors of RNA synthesis. It was assumed that through a mechanism similar to that in vitro lysine-rich histone may interfere with the in vivo synthesis of protein necessary for learning with the resultant changes in acquisition and/or latency.

I.c. injection of arginine-rich histones and bovine serum albumin served as controls in 2 groups of rats. Prolonged administration of either histone fractions or bovine serum albumin did not produce any toxic side effects, nor did they influence the performance of the animals. These results, based on careful control of experimental conditions, confirm those who reject the notion that administration of RNA has any relevance to learning or memory[12].

Zusammenfassung. RNA aus Hefe, RNA-Hydrolysat, Tubercidin und mit Lysin angereichertes Histon, i.c. oder i.p. injiziert, hatten keine Wirkung auf die Lernfähigkeit von Ratten. Intracerebral injiziertes ^{14}C-RNA verbreitete sich schnell über das ganze Gehirn und verschwand relativ schnell wieder. Nach i.p. Injection von ^{14}C-RNA konnte jedoch ein geringer Betrag von ^{14}C im cerebralen RNA gefunden werden.

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[10] The daily intracerebral injection of TU must not exceed 2.5 µg of the antibiotic. Above that level TU produces toxic side effects which interfere with the behavioral testing. However, even at this level the period of injection should not exceed 7 days since the total cumulative effect of over 17 µg TU will decrease survival to 60% with the avoidance response of survivors dropping from an average of 67% to about 30%. With 40 µg total TU injected the survival drops to 10% with an avoidance response of about 7%. (These poor performance responses must be attributed to illness rather than a specific effect on learning ability.)

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Antagonism of Terramycin on Action of *Bacillus thuringiensis* 'Exotoxin' in *Drosophila melanogaster*

The heat-stable 'exotoxin' (ET) produced by certain serotypes of *Bacillus thuringiensis* has the chemical nature of a complicated nucleotide, which in addition to adenine, ribose, and phosphate[1-3] contains the unusual constituents glucose and allomucic acid[4-6]. The substance has the molecular weight of 755 and has been named thuringiensis A. The corresponding γ-lactone is nontoxic and is called thuringiensis B[6]. Because of the chemical nature of ET, the hypothesis had been forwarded that it might act as an antimetabolite of the nucleic acid metabolism[2]. This hypothesis has since been verified to some extent by Czech authors who found reduced incorporation of orotic acid and cytidine into the liver RNA of mice[7] and inhibition of DNA-dependent RNA polymerase in *Escherichia coli*[8]. The following report of an antagonism of terramycin and ET in *Drosophila* may throw further light on the mechanism of action of ET in an insect.

The toxicity of ET in *Drosophila* reared on a yeast free medium and the antagonistic effect of yeast and certain yeast extracts has been described earlier[9,10]. For toxicological data on terramycin and information on the rearing and assay method used in the present work, the reader should consult another paper[11]. Terramycin allows complete survival of *Drosophila* on a medium containing up to 250 ppm of the antibiotic, whereas concentrations above 600 ppm cause significant mortality (LC_{50} = 0.115%). In the present study the concentrations of terramycin have therefore been restricted to 200 ppm or less. However, even sublethal concentrations of 100 to 250 ppm of terramycin reduce the rate of larval growth and delay the time of pupation by 16 to 50%. The influence of terramycin on the toxic action of ET was investigated in combinations where different concentrations of both the antibiotic and ET were added to the rearing medium of the larvae. The ET preparation used was a prepurified[2] culture medium of *B. thuringiensis var. thuringiensis*. Homologization with a sample of pure ET (kindly furnished by Dr. R. P. M. BOND of the Milstead Laboratory, Sittingbourne, England) indicated that our preparation had an ET concentration of 100 ppm. Total mortality was recorded for ET alone and the combinations. The data were subjected to probit analysis, using 2 computer programs[12].

Except for very low doses, ET alone gave straight dose-mortality curves with a slope of 4.5 to 5.0, i.e. similar to lethal doses of terramycin alone[11]. Correspondingly the dose-mortality curves of the combinations of ET with terramycin had the same slope (Figure). However, the LC_{50} values for the combinations are higher than the LC_{50} of ET alone, indicating an antagonistic action of the antibiotic.

The antagonistic effect of different concentrations of terramycin can be determined if the factor of the parallel displacement of the curves, i.e. the potency (P) is calculated[12], P of ET alone having the value of 1. The LC_{50}