little of the histamine injected into the cerebral ventricles, and its metabolites, was absorbed into the blood stream. This is in agreement with earlier findings obtained with somewhat different techniques(8,9).

*Discussion.* The present results support the view that histamine administered to the brain is rapidly methylated by the enzyme histamine-N-methyl transferase(10), and then, in part, further oxidized to methylimidazoleacetic acid, whereas histamine formed in the brain from histidine is less rapidly methylated(3). Such a difference in the extent of methylation between "exogenous" and endogenous" histamine was not observed by Bjurö, Westling and Wetterqvist(11) in the urine of intact rats after subcutaneous injections of radioactive histamine and histidine, respectively. This suggests that, disregarding possible species differences, the metabolism of histamine in the brain is quantitatively different from the metabolism of histamine in the rest of the body. This may be related to the findings that histamine penetrates poorly, or not at all, from the blood into the brain(12), and that histamine administered to the brain, and its metabolites there, are absorbed into the blood stream only to a very small extent, as mentioned above. The present results in conscious cats agree fairly well with observations on the metabolism of histamine and histidine administered to the brain by ventricular perfusion in anaesthetized cats (3,4).

*Summary.* $C^{14}$-histamine and $C^{14}$-histidine were injected into the cerebral ventricles of conscious cats. After one to two hours $C^{14}$-histamine and its metabolites in the brain were determined. The formation of histamine from histidine, and methylhistamine from histamine, were demonstrated. The results indicate that in the brain "exogenous" histamine is more rapidly methylated than "endogenous" histamine.

1. Harris, G. W., Jacobsohn, D., Kahlson, G., *Ciba Foundation Colloquia on Endocrinology,* 1952, v4, 186.

2. Adam, H. M., *Regional Neurochemistry,* S. S. Kety, J. Elkes, ed., Pergamon Press, Oxford, London, New York, Paris, 1961, p293.

3. White, T., *J. Physiol.,* 1960, v152, 299.

4. ———, *ibid.,* 1961, v159, 191.

5. Feldberg, W., Sherwood, S. L., *ibid.,* 1953, v120, 3P.

6. White, T., *ibid.,* 1961, v159, 198.

7. Schayer, R. W., *Brit. J. Pharmacol.,* 1956, v11, 472.

8. White, T., *Experientia,* 1963, v19, 43.

9. Bhawe, W. B., *J. Physiol.,* 1958, v140, 169.

10. Brown, D. D., Tomchick, R., Axelrod, J., *J. Biol. Chem.,* 1959, v234, 2948.

11. Bjurö, T., Westling, H., Wetterqvist, H., *Arch. int. Pharmacodyn.,* 1963, v144, 337.

12. Halpern, B. N., Neveu, Th., Wilson, C. W. M., *J. Physiol.,* 1959, v147, 437.

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## Evidence for Cholinergic Transmission at a Central Synapse.* (29062)

D. T. FRAZIER AND L. L. BOYARSKY (Introduced by L. D. Carlson)
*Department of Physiology and Biophysics, University of Kentucky Medical Center, Lexington*

A large amount of evidence exists involving acetylcholine as a synaptic transmitter in the central nervous system(1). The experiments reported here employ the hemicholinium compound HC-3 to test the cholinergic properties of synapses in the cuneate nucleus, the first relay nucleus in the afferent pathway for the sensation of touch. HC-3, first synthesized and investigated by Schueler(2), has been shown to interfere with the synthesis of acetylcholine in sympathetic ganglia and minced brain(3). It has also been demonstrated that choline can antagonize the effects of HC-3(2). Several workers have

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shown that HC-3 acts by interfering with choline uptake(4,5,6).

The present experiments were designed to examine the effects of HC-3 on transmission through the cuneate nucleus by employing the evoked potential of the nucleus as an index of transmission. If HC-3 interferes with acetylcholine synthesis and acetylcholine is the chemical transmitter, then HC-3 should diminish or abolish the potentials, once the stores of acetylcholine have been depleted by sufficient stimulation. It would also be anticipated that choline should inhibit the action of HC-3 in the depressed nucleus and restore transmission.

*Materials and methods.* Nine cats were used as experimental animals. The radial nerve and cuneate nucleus were exposed on both sides. The radial nerve was stimulated with a Grass S-4 stimulator and isolation unit. The stimulating pulse was a square wave 0.1 msec in duration. Tungsten microelectrodes, sharpened electrolytically and insulated with Insl-X, were inserted into the cuneate nucleus. The slow, positive potentials of the cuneate nucleus were amplified with Grass P-4 preamplifiers and a Tektronix 502 double-beam oscilloscope and photographed with a Grass camera. The amplitude of the potentials was measured directly from the photographs. Drugs were injected into the femoral vein. The compound HC-3† and choline, injected as choline chloride (Nutritional Biochemicals Co.) were given in mammalian Ringer's solution.

*Results. Effect of HC-3 following stimulation.* In the first group of experiments, high frequency stimulation (50/sec) was applied to one radial nerve following HC-3 injection. A typical result of the injection of 200 $\mu$g/kg is shown in Fig. 1. The radial nerves of both sides were stimulated continuously at a low frequency (0.5/sec). During the intervals indicated by the lettered blocks, additional stimulation at 50/sec was applied to the left radial nerve for one-minute periods. Following each period of stimulation, a fall in the potential of the left cuneate nucleus occurred. Between the second and third periods of stimulation, a second, identical dose of HC-3 was given. When stimulation was then given during period C, a somewhat greater fall in

† We are indebted to Dr. F. Schueler for a generous supply of HC-3.

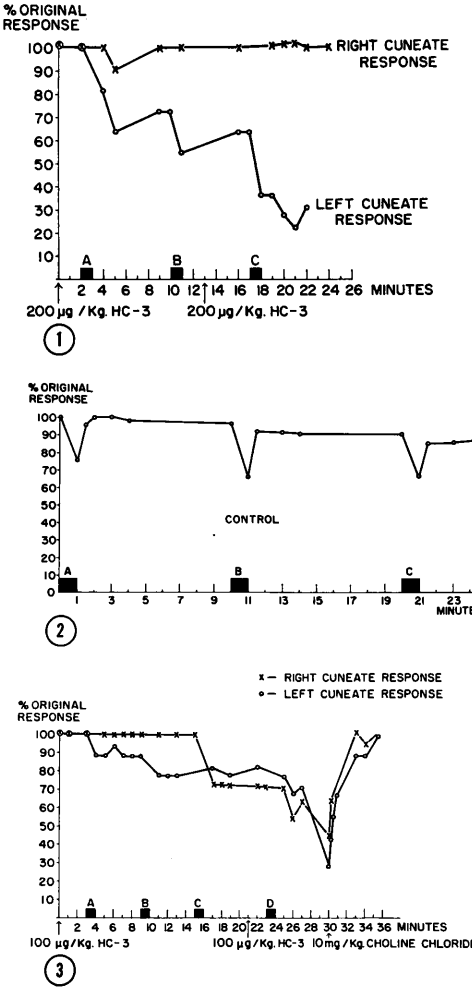


FIG. 1. Effect of stimulation on evoked potentials of cuneate nucleus following HC-3 injection. Ordinate indicates amplitude of evoked potential as percent initial value. Both radial nerves were stimulated continuously at 0.5/sec. During periods indicated by A, B, C, the left radial was stimulated at 50/sec.

FIG. 2. Effect of stimulation on right cuneate response of cat not treated with HC-3. During periods A, B, C, the right radial nerve was stimulated at 50/sec.

FIG. 3. Effect of choline chloride on transmission through depressed cuneate nucleus. During A and B, the left radial nerve was stimulated at 50/sec; during C and D, the right was stimulated at 50/sec. Choline chloride was injected 30 min following the beginning of experiment.

potential took place than following periods A and B. A slow recovery of the potential was apparent after each period of stimulation. The right radial nerve was stimulated continuously at the low rate of 0.5/sec. The potentials recorded from the right cuneate nucleus showed no significant decrease throughout the experiment. Seven experiments were performed in which a persisting decrement in response of 25 ± 5% occurred following a single dose of HC-3 and high frequency stimulation.

Another pertinent control concerning the effect of stimulation alone is shown in Fig. 2. The right radial nerve of an animal which had not been treated with HC-3 was stimulated continuously at 0.5/sec and the responses of the ipsilateral cuneate nucleus to 3 one-minute periods of 50/sec stimulation were recorded. As a result of each period of high frequency stimulation there is a temporary diminution in the amplitude of the potentials for one to two minutes followed by a rapid recovery to an amplitude of responses slightly lower than before stimulation. After 3 periods of stimulation, there is a reduction of only 15%.

*Effect of choline chloride.* A number of experiments were carried out to investigate the ability of choline chloride to reverse the depression caused by HC-3. Fig. 3 shows the results obtained in a typical experiment. Both radial nerves were stimulated continuously at a frequency of 0.5/sec. Following the injection of HC-3, the left radial nerve was stimulated twice during periods A and B; the right radial nerve was stimulated during periods C and D. Between periods C and D, injection of a second dose of HC-3 resulted in a precipitous fall in the response of both nuclei. When the amplitude of the response had fallen to about 30% of the initial value in each nucleus, choline chloride (10 mg/kg) was injected. Within 4 minutes, the amplitude of the responses in both nuclei had returned to values obtained at the beginning of the experiment. Four experiments were performed in which choline restored potentials which had been depressed by HC-3 injection and high frequency stimulation. In one experiment, choline failed to restore the potentials. Choline chloride (11 mg/kg) injected 20 minutes before HC-3 (575 μg/kg) prevented the usual effects of high frequency stimulation in one experiment.

*Discussion.* These experiments demonstrate that HC-3 causes a depression of response of cuneate neurones if HC-3 is followed by a period of high frequency stimulation of the afferent input to the nucleus. This finding agrees with the hypothesis that transmission through many cuneate synapses is the result of the release of acetylcholine. In the experiments reported here, high frequency stimulation presumably caused a decrease in the amount of acetylcholine stored at the presynaptic terminals. Resynthesis of acetylcholine could not occur in the terminals, either because choline was prevented from entering the nerve fiber, or because HC-3 substituted for choline at the storage sites and thereby prevented synthesis directly. Both these hypotheses have been suggested by MacIntosh as possible explanations for the effect of HC-3 on the superior cervical ganglion(3). The failure of low frequency testing stimuli to cause depression of the potentials is evidence that a postsynaptic action of HC-3 is not taking place.

The experiments in which choline restored transmission in nuclei which had been depressed suggest that choline and HC-3 are competitors for a site which is critical for the synthesis of acetylcholine. A similar competitive action of choline and HC-3 has been reported at the superior cervical ganglion(3) and at the neuromuscular junction(7).

*Summary.* Intravenous injection of HC-3 resulted in depression of potentials in the cuneate nucleus of the cat following high frequency stimulation of the radial nerve. No depression followed low frequency continuous stimulation. Choline chloride administered after HC-3 and high frequency stimulation had resulted in depression, restored transmission through the nucleus. The results are consistent with the presence of cholinergic synapses in the cuneate nucleus.

1. Hebb, C. O., Krnjvic, K., *Neurochemistry,* Elliot, K. A. C., Page, I. S., Quastel, J. H., Eds.,

Charles C Thomas, Springfield, 1962.
2. Schueler, F., *J. Pharmacol. Exp. Therap.*, 1955, v115, 127.
3. MacIntosh, F. C., Birks, R. I., Sastry, P. B., *Nature*, 1956, v178, 1181.
4. ———, *Neurology*, 1958, v8, (Supplement), 90.
5. Gardiner, J., *J. Physiol.*, 1957, v138, 13P.
6. MacIntosh, F. C., *Fed. Proc.*, 1961, v20, 562.
7. Reitzel, N. L., Long, J. P., *Arch. Intern. Pharmacodynamie*, 1959, v119, 20.

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## Response of Normal and Immune Histiocytes of Various Animal Species to Infection by *Brucella melitensis.** (29063)

SANFORD S. ELBERG, PATRICIA MASCARENHAS AND JACOB FONG
*Department of Bacteriology, University of California, Berkeley*

One has attempted to observe the relation which exists between peritoneal histiocytes and *Brucella melitensis* after uptake of the latter by normal and immune cells *in vitro*. To this end maintenance and growth of the histiocytes are essential for relatively long periods in order to be certain of the vitality of the host cell, although the end-point of the experiment is available within 3-4 days of incubation. Indeed the major outlines of the response of histiocytes to the cytotoxicity of the brucellae are manifest within the first 24-hour period of incubation, after which further degeneration or continued survival followed by multiplication is principally a function of the relative virulence of the brucellae.

The data will show that peritoneal histiocytes from rabbit, monkey and guinea pig do not differ in their cytotoxic response. This response is non-specific in terms of the species of brucella being studied for immunization and challenge infection.

*Methods.* Histiocytes were harvested and treated as previously described by Elberg *et al*(1). The cells were exposed to *B. melitensis*, strain Rev I, which had been grown on Albimi agar and washed 3 times in modified Tyrode solution.† A ratio of 10 bacteria: 1 monocyte was used for parasitization. The cells were then cultivated in either Mackaness or Sykes-Moore microchambers. Normal cells were cultivated in Tyrode + 40% normal rabbit serum; immune cells were grown in 40% immune serum in Tyrode solution. Samples were taken at 3, 24, 72, 120, and 168 hours.

*Results.* Maintenance was well demonstrated in the chamber cultures. The customary degeneration after parasitization of histiocytes, derived from normal animals, and due to the cytotoxic action of the brucellae, is seen in the data of Table I which presents a sample from many animals and chambers giving identical results.

A comparison of Hartley strain of guinea pig and *Cynomolgus phillipinensis* histiocytes

TABLE I. Survival and Multiplication of Rabbit Peritoneal Histiocytes Following Parasitization by *B. melitensis*, Strain Rev I.*

| | 0 hr | 24 hr | 48 hr | 96 hr |
|---|---|---|---|---|
| Non-parasitized (a) | 559 | 561 | 592 | 896 |
| normal cells (b) | 637 | 639 | 675 | 920 |
| Parasitized (a) | 631 | 461 | 458 | 503 |
| normal cells (b) | 833 | 615 | 601 | 670 |
| Non-parasitized (a) | 396 | 394 | 400 | 700 |
| immune cells (b) | 436 | | 443 | 658 |
| Parasitized (a) | 452 | 450 | 450 | |
| immune cells (b) | 489 | 493 | 501 | 891 |

* Attenuated vaccine strain.

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† Modified Tyrode Solution: *Solution A:* NaCl, 7.7 g; KCl, .2 g; $MgSO_4$ (anhydrous) .1 g; Dextrose, 4.0 g; $NaH_2PO_4$, .05 g; Water, 750 ml. *Solution B:* $NaHCO_3$, .5 g; Water, 25 ml. Double-distilled water is used. The solutions are autoclaved separately and combined when *cool*. If an indicator is desired, .1 ml of phenol red (La Motte Solution, 1%) is added to Solution A before autoclaving.