tained an ample methyl supply (0.16% choline). When the methyl supply was suboptimal (0.08% choline) both vit. $B_{12}$ and folacin were essential. A possible explanation of these data and recent observations(8,12) is that folacin functions primarily in methyl synthesis and vit. $B_{12}$ functions in either the transfer or utilization of labile methyl.

For the methylation of homocystine to methionine, betaine is at least as effective a methyl donor as choline. When animals are fed a diet deficient in choline, 3 moles of betaine are required to replace 1 mole of choline. This substantiates previous reports (13,14) that betaine replaces choline *per se* mainly by virtue of supplying one methyl group per mole of betaine.

Vit. $B_{12}$ and folacin were essential for the synthesis of choline and methionine from aminoethanol, homocystine, and a limited supply of betaine. Under the experimental conditions employed, the amount of biological synthesis of labile methyl or of the utilization of methyl from methanol, or synthesis of the entire choline moiety, was insufficient to meet the entire choline requirement necessary for the prevention of renal hemorrhage in the *weanling rat.*

*Summary.* The role of vit. $B_{12}$ and folacin in the biosynthesis of choline and methionine has been studied and the following conclusions are warranted: 1. In the presence of adequate methyls (from choline), vit. $B_{12}$ alone was as effective for methionine synthesis as when combined with folacin. When fed alone, folacin was ineffective. 2. In the presence of a limited supply of methyl groups, both folacin and vit. $B_{12}$ were essential for the normal development of weanling rats. 3. Vit. $B_{12}$ and folacin were essential for the synthesis of choline and of methionine from aminoethanol, homocystine, and a limited supply of betaine. 4. As a methyl donor for the synthesis of methionine from homocystine, betaine was at least as effective as choline, mole per mole. 5. Under the experimental conditions employed, the rate of synthesis of the methyl group or utilization of methanol for the methylation of homocystine to methionine and aminoethanol to choline was not fast enough to support normal development of *weanling rats.*

13. Schaefer, A. E., Strength, D. R., and Salmon, W. D., *J. Nutrition,* 1951, in press.

14. Stetten, D., *J. Biol. Chem.,* 1941, v140, 143.

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## Changes in Acetylcholine Content of the Brain During Exposure to Cold. (18883)

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That acetylcholine (ACh) may play a role in the temperature regulatory mechanism of the mammalian organism and that its effect might be modified by changes in body temperature are suggested by the findings of several investigators. Thus, White(1) in 1929 reported that atropine (an anticholinergic drug) in moderate or excessive amounts produced a marked hyperthermia in infants and children. On the contrary, Burn and Dutta(2) showed that the body temperature of mice is lowered after atropine administration. Grosse-Brockhoff(3) reported therapeutic effects of atropine in hypothermia by diminish-

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1. White, P. J., *Am. J. Dis. Child.,* 1929, v37, 745.

2. Burn, J. H., and Dutta, N. K., *Nature,* 1948, v161, 18.

3. Grosse-Brockhoff, F., German Aviation Medicine in World War II, U. S. Govt. Print. Office, Wash., D. C., v2, ch. VIII-E, 1950.

ing excessive vagal influences and increasing the vascular tonus. Tournade and Curtillet (4) found the lethal dose of ACh to be considerably reduced in cold animals. It seems likely that the cardiovascular system is primarily involved in this change. Noell(5) has shown, however, that the brain also undergoes changes. He observed certain alterations in the electrical response of the cerebral cortex in rabbits whose temperature had been depressed to 28° to 34°C by submersion, which were similar to those obtained on uncooled rabbits receiving low concentrations of eserine intravenously. These experiments suggested that an investigation of the ACh content of tissues might show an increase in animals exposed to cold. The following study reports the results of such measurements on the brain.

*Experimental.* Free and total (free plus bound) ACh concentrations in rat brain were determined by the leech method described by MacIntosh and Perry(6). Rats were decapitated immediately on removal from the cage and the brain tissue anterior to the anterior quadrigemina removed, weighed, and homogenized in eserine-Locke's solution.‡ A Potter-Elvehjem homogenizer was employed. The maximum elapsed time between decapitation and immersion of the excised brain was 30 seconds. We found the leech muscle extremely sensitive to hydrogen ion concentration, particularly with respect to relaxation between test contractions. Since satisfactory relaxations were observed at pH 7.0, all solutions were adjusted to within 0.05 pH§ unit of this value before immersion of the leech muscle. No muscle was used unless it contracted at least 7 cm (as read at the end of a lever arm 20 cm from the fulcrum) against a force of 8.5 g cm, when exposed for 3 minutes to acetylcholine concentration of 0.1 μg per ml. The homogenates were diluted to produce contractions equivalent to those observed with ACh standards of 0.03 to 0.05 μg per ml. A blank was determined on each sample by destroying the ACh with alkali and boiling prior to analysis.

TABLE I. Brain ACh Concentration of Control and Chilled Rats (−12°C for 2 Hr).

| Group | No. rats | ACh (μg/g wet wt) Free | $SE_M$* | Total | $SE_M$* |
|---|---|---|---|---|---|
| Control | 29 | 1.15 | .07 | 1.99 | .11 |
| Cold | 30 | 1.54 | .06 | 2.45 | .18 |
| P | | <.01 | | .04 | |

* Standard error of mean $= \pm \frac{\sigma}{\sqrt{N}}$.

The accuracy of the assay was determined by recovery studies using the brains of 15 male rats selected without regard to weight, age, or strain. The brains were analyzed as described above. To an aliquot portion of the analyzed homogenate, known quantities of ACh were added and the recoveries calculated. Recoveries averaged 99 ± 11%.

Male, Sprague-Dawley rats of the same age (6 months) and weight (250 to 300 g) were selected to determine changes induced by acute cold exposure. Half the animals were exposed to a temperature of −12°C for 2 hours, at which time their brain ACh was determined individually, as already described. The other half remained at room temperature (25°C), but were otherwise treated identically. None of the animals died from the cold, but the average body temperature had fallen to 15°C at the end of the exposure period. A significant increase in both free and total ACh of the cold animals is shown in Table I.

*Discussion.* A possible explanation for the observed increase in ACh after cold exposure is the reduced activity of the rat under these conditions. An inverse relationship has been shown to exist between brain ACh and activity. Thus Richter and Crossland(7) reported a rise of 40% above normal levels during anesthesia, and an even greater increase in deep narcosis has been claimed by other

4. Tournade, A., and Curtillet, E., *Compt. Rend. Soc. de Biol.*, 1936, v121, 505.

5. Noell, W. K., unpublished data.

6. MacIntosh, F. C., and Perry, W. L. M., Methods in Medical Research, Year Book Pub. Co., Chicago, 1950.

‡ NaCl, 6.43 g; glucose, 0.715 g; $NaHCO_3$, 0.36 g; KCl, 0.30 g; $CaCl_2$, 0.07 g. Dilute to 1 liter and add 0.42 mg eserine sulfate.

§ Determined with Cambridge pH Meter, Model R.

7. Richter, D., and Crossland, I., *Am. J. Physiol.*, 1949, v159, 247.

workers(8). Another possibility to be considered is a change in the relative rates of synthesis and hydrolysis of ACh produced by a lowered body temperature. Further studies are necessary to decide whether these or other alterations are responsible for the ACh increase.

*Summary.* Exposure to −12°C for 2 hours significantly increased both the free and total acetylcholine concentration of rat brain.

The statistical help given by Dr. William F. Taylor, Department of Biometrics, is acknowledged.

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## Isolation from Human Sera in Egypt of a Virus Apparently Identical to West Nile Virus.* (18884)

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This report describes the isolation from human sera collected in Egypt, of 3 strains of a virus characterized by the capacity to produce encephalomyelitis in monkeys, mice and hamsters, and immunologically related to West Nile virus. The circumstances under which these virus strains were isolated were as follows. In the course of an "antibody survey," carried out in July 1950, one of us (J.R.P.), collected serum from 251 Egyptians living within a Rural Health District known as Markaz Callub, established by the Ministry of Public Health with the cooperation of the Rockefeller Foundation, and located some 30 km. north of Cairo.‡ Most of the Egyptians were children and most of them were attending a Dispensary. Apparently at least 3 of the children happened to be in stages of viremia at the time they were bled, for it was from their sera that the strains were isolated.

All 3 strains were subsequently found to be antigenically related to each other and to the West Nile virus. The latter virus was isolated at the Yellow Fever Research Institute in Entebbe from a human serum obtained in 1937 in Uganda, about 2000 miles south of Cairo(1), but it has not been recovered in nature since then. However, serological evidence obtained by Smithburn(2) pointed to widespread human infection with this virus in Central Africa, with 1 to 46% of the various population groups studied there yielding positive tests for neutralizing antibodies to West Nile virus. In like manner evidence has been obtained in the present study to show that antibodies to the newly isolated Egyptian virus are present in a very large percentage of

* Laboratory work carried out in New Haven under the auspices of the Commission on Virus and Rickettsial Diseases, Armed Forces Epidemiological Board, Office of the Surgeon General, U. S. Army, Washington, D. C.

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2. Smithburn, K. C., and Jacobs, H. R., *J. Immunol.*, 1942, v44, 9.