taneously with rate of uptake of isotopically labelled glycine and lysine into the protein of rat liver slices. The synthetase system increased by 40% in the hyperthyroid state. Incorporation of labelled amino acids, on the other hand, was uninfluenced in the same situation.

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1. Borsook, H., *J. Cell. Comp. Physiol.*, 1956, v47, 35, Supplement 1.
2. Cohen, P. P., and McGilvery, R. W., *J. Biol. Chem.*, 1946, v166, 261.
3. ———, *ibid.*, 1947, v169, 119.
4. ———, *ibid.*, 1947, v171, 121.
5. Watts, D. T., and Gourley, D. R. H., PROC. SOC. EXP. BIOL. AND MED., 1953, v84, 585.

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Restraint Hypothermia and I^{131} Uptake by Rat Thyroid.* (23042)

ROSCOE G. BARTLETT, JR. (With the technical assistance of Henry Mengoli)
Nat. Inst. of Arthritis and Metabolic Diseases, N.I.H., Bethesda, Md. and Dept. of Physiology, College of Medicine, Howard Univ., Washington, D. C.

Oxygen consumption in rats restrained in the cold is initially higher than in nonrestrained animals at the same environmental temperature. Soon, however, both body temperature and oxygen consumption fall in restrained animals while they remain unchanged in nonrestrained controls(1). Since not all energy expenditure in muscle activity appears as heat, the initially higher oxygen consumption in the restrained, struggling animals need not represent an increased heat production if the basal heat production was so lowered that the total heat production was inadequate for maintaining normothermia. In point of fact, cold and restraint lower liver nonprotein sulfhydryl compounds(2-4) which might indicate some defect in intermediary metabolism and a consequently lowered basal heat production. None of these data, however, clearly distinguish whether the falling metabolism is the cause or the effect of the hypothermia. Since rate of iodine uptake is believed to reflect metabolic activity of the thyroid gland, chief regulator of basal body metabolism, the present experiments on thyroid uptake of I^{131} were undertaken to explore further the effect of restraint in the cold on basal metabolism.

Methods. In studying rate of iodine uptake by the thyroid as affected by restraint and cold, 175-225 g adult male Sprague-Dawley rats were injected at zero hours with 5 μc I^{131} (as KI), immediately divided into 2 groups (one of which was restrained in loose-fitting wire mesh cylinders, flattened on the underside and closed at the ends) and put into a cold room (5° ± 2°C). Then at 1, 2 and 4 hours equal numbers of restrained and nonrestrained animals were killed with ether and their thyroids removed to glass micro slides and air dried overnight. I^{131} activity of the dried glands was determined with a scintillation counter from 10 one-minute (later one 3-minute) counts per gland pair. This was usually done on the day following death, but in any case the measurement was corrected for background (avg 255 cpm) and for decay from zero time. Six animals in each of the 6 groups were used on each of 10 days, making a total of 360 rats. In many of the 180 restrained animals colonic temperature was also recorded at intervals during the 4 hour period. To ascertain the relation between body temperature and thyroid iodine uptake in restrained and nonrestrained animals, thyroid I^{131} activity in 25 of the 4-hour restrained rats (average colonic temperature 17.8°C) was compared with that in 25 extra injected nonrestrained controls in which the

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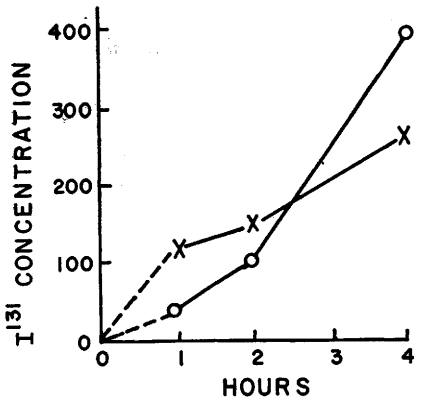

FIG. 1. Thyroid I^{131} concentration (expressed as counts/min.) in restrained (×) and nonrestrained (○) rats at 5° ± 2°C.

body hair had been wetted before 4 hours of cold exposure. This treatment brought their average colonic temperature down to 17.3°C. Thyroid iodine uptake could not be followed *in vivo,* because of the masking effect of I^{131} in blood and tissue fluids adjacent to the thyroid and because the struggling of the animals too frequently shifted the thyroid gland relative to the sensing element. The also potentially desirable technic of determining the rate of release of radioactive thyroxin could not be used in these short term experiments either, because in one hour only 5% of the labelled hormone was lost, an amount comparable to the experimental error and large in comparison with the differences between control and experimental animals.

Results. Fig. 1 gives mean thyroid I^{131} counts, with standard errors including both counter and background variations, for restrained and nonrestrained rats. Total uptake was significantly higher in the restrained animals at one hour ($p<.05$) and lower at 4 hours ($p<.05$). The mean colonic temperature of the restrained rats was 18.2°C at 4 hours.

The thyroid counts for 25 restrained 4-hour rats, of mean colonic temperature 17.8°, averaged 251 ± 25, whereas those in 25 nonrestrained but wetted controls, with mean temperatures of 17.3°, averaged 104 ± 18. The difference was significant ($p<.01$).

Discussion. The difference in I^{131} uptakes might be ascribed to more rapid dissipation of glycogen in the restrained struggling rat as compared to the control, with the subsequent fall in temperature and metabolic rate being due to diminished reserves. This agrees with the observation that restrained rats which struggle more become the more hypothermic (5). It is not in agreement, however, with the data showing greater thyroid I^{131} uptake in restrained (struggling) rats than in equally hypothermic nonrestrained (quiet) controls.

The significantly higher thyroid I^{131} count of restrained rats, at the one-hour measurement, as compared to the control animals, reflects a much greater rate of accumulation of I^{131} (Fig. 1). This early increased rate of I^{131} accumulation in the restrained rat, however, gives way to a lesser rate of uptake as compared to the control, for at the 4-hour comparison the control thyroid I^{131} count was significantly greater than for the restrained. These changes correlate well with the rate of oxygen uptake in the cold where restrained rats initially have a greater rate, and after approximately 1½ hours, a progressively lesser rate than nonrestrained controls(1). Although the response of the thyroid is usually regarded as being too slow to account for an increased O_2 consumption occurring in one hour or less, strong presumptive evidence favoring such a hypothesis is the observation by Booker *et al.*(6) that 1½ hours of cold exposure to both normal and adrenalectomized mice results in a 100% increase in the oxygen consumption of live homogenate.

The parallel falls in body temperature, rate of thyroid iodine uptake and rate of O_2 consumption in restrained rats exposed to between 2 and 4 hours of cold give no clue as to whether falling heat production causes the hypothermia or the reverse. The fact that both the rate of I^{131} uptake and O_2 uptake are above corresponding control values at one hour is not conclusive either, for both these may have been caused by the greater struggling of the restrained rats. However, the fact that thyroid iodine uptake was much higher in restrained rats 4 hours in the cold than in nonrestrained but equally hypothermic controls indicates that restraint causes hypothermia by some mechanism other than

depression of heat production. The drop in body temperature of the restrained rat, in fact, appears to be the cause of the drop in oxygen consumption (metabolism), for if the reverse were true the I^{131} uptake (thyroid metabolism) should have been higher in the nonrestrained than in the restrained animals.

Summary. Thyroid I^{131} uptake by restrained rats was higher at one hour, and lower at 4 hours, than that of nonrestrained control animals. The lower the body temperature of the restrained animals at 4 hours, the lower the I^{131} uptake by the thyroid. Restrained hypothermic rats had a significantly greater thyroid uptake of I^{131} than equally hypothermic nonrestrained animals. It is concluded that hypothermia of restrained animals can not be ascribed to depressed basal metabolism.

1. Bartlett, R. G., Jr., Bohr, V. C., and Inman, W. I., *Can. J. Biochem. and Physiol.*, 1955, v33, 654.

2. Bartlett, R. G., Jr., and Register, U. D., PROC. SOC. EXP. BIOL. AND MED., 1953, v83, 708.

3. ———, *ibid.*, 1954, v86, 397.

4. Register, U. D., and Bartlett, R. G., Jr., *ibid.*, 1954, v86, 836.

5. Bartlett, R. G., Jr., Bohr, V. C., Foster, G. L., Miller, M. A., and Helmendach, R. H., *ibid.*, 1956, v92, 457.

6. Booker, W. M., DaCosta, F. M., Mitchell, S. Q., and Shelton, M. L., *Endocrinology*, 1956, v59, 317.

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Association of Mouse Pathogenic Strain of Echo Virus Type 9 with Aseptic Meningitis.* (23043)

DONALD M. MCLEAN† AND JOSEPH L. MELNICK

Department of Pathology, University of Cambridge, England, and Section of Preventive Medicine, Yale University School of Medicine, New Haven, Conn.

Echo viruses are a new group of agents which have been isolated from stools of aseptic meningitis cases and of normal children(1). Although one of the 14 antigenic types, echo-6, has repeatedly caused aseptic meningitis(2-5), the etiological relationship of most types to human disease remains obscure. The present paper offers evidence that echo-9 virus has caused one epidemic of typical aseptic meningitis near Cambridge, England, during 1955, and two epidemics of this syndrome with milder symptoms in other nearby areas during 1956.

Methods. Virus isolations and antibody studies were performed in cultures of human amnion cells(6) which had been grown for the first 3 days in medium containing 20% human serum and 0.5% lactalbumin hydrolysate in Gey's balanced salt solution followed by 3 days in medium containing 10% inactivated rabbit serum and 0.5% lactalbumin hydrolysate in balanced salt solution. Immediately prior to inoculation, the cell sheets were washed twice in salt solution and a maintenance medium, containing 5% rabbit serum and 0.25% lactalbumin hydrolysate in salt solution, was added. Uninoculated cultures remained intact in maintenance medium for 10 days. Monkey kidney (MK) cell cultures were also used. Those in Cambridge were kindly provided by Dr. J. O'H. Tobin, while those used in New Haven were prepared by methods described elsewhere(7). Virus isolations were attempted from feces obtained from patients between 2 and 7 days after onset of illness. In the third outbreak, virus isolation was attempted from both throat washings and rectal swabs. Samples of serum were collected from each patient between 2 and 7 days and again between 12 and 21 days after onset of illness. Neutralization tests on paired sera were performed on serial

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† Harrison Watson Student, Clare College, Cambridge, England; present address, Commonwealth Serum Laboratories, Melbourne, Australia.