venous differences in concentration of Fractions 1 and 3 plus 4 in Exp. II suggests that they were secreted at a low rate. However, when arterial blood to which 17-hydroxycorticosterone (Exp. II, 1000 μg; Exp. III, 1500 μg) and corticosterone (500 μg in each experiment) had been added was analyzed and the concentrations of the various fractions compared to those of the control samples of arterial blood (Table II) a small positive difference in concentrations of Fractions 1 and 3 plus 4 of Exp. II was found. The possibility that these fractions may be formed by the degradation of one or more of the major fractions during processing cannot be ruled out.

3) *Recovery of 17-hydroxycorticosterone and corticosterone added to arterial blood.* In Table II are presented the results of the recovery studies. 17-Hydroxycorticosterone added to blood (100 μg/100 ml in Exp. I and 150 μg/100 ml in Exp. II) was recovered in 96% and 86% yields, respectively. Fifty μg/100 ml of corticosterone was added to blood in both experiments and was recovered in yields of 60% and 84%, respectively. Other than the very slight absolute increase in the concentrations of Fraction 1 and Fraction 3 plus 4 in Exp. II (see above) there is no evidence of change in the pattern of steroids. These experiments indicate that none of the major fractions which have been described in adrenal venous blood is an artifact derived by degradation of the major constituents during extraction and chromatography.

Summary. Concentrations of steroids in arterial and venous blood of the adrenal have been determined. Those steroids whose concentrations in the blood are increased by passage through the gland are considered to be adrenal secretory products. Three steroids are consistently of adrenal origin: 17-hydroxycorticosterone, Fraction 8, and corticosterone. In 2 of 3 experiments 11-desoxy-17-hydroxycorticosterone was contributed to the blood by the adrenal gland. A steroid less polar than corticosterone (Fraction 13) also appeared to be an adrenal secretory product in 2 of 3 experiments. Five substances (Fractions 2, 5, 7, 9, and 10) are clearly not secreted by the adrenal gland, since they were found in the same concentrations in arterial blood as in adrenal venous blood. The status of 4 substances (Fractions 1, 3, 4, and 14) as adrenal secretory products has not been established. 17-Hydroxycorticosterone and corticosterone, when added to arterial blood, are recovered without substantial alteration in the pattern of steroids present in such blood.

1. Farrell, G. L., and Lamus, B., PROC. SOC. EXP. BIOL. AND MED., 1953, v84, 89.

2. Burton, R. B., Zaffaroni, A., Keutmann, E. H., *J. Biol. Chem.*, 1951, v188, 763.

3. Farrell, G. L., *Fed. Proc.*, 1954, v13, 42.

4. Nelson, D. H., and Samuels, L. T., *J. Clin. Endocrinol. and Metab.*, 1952, v12, 519.

5. Sweat, M. L., and Farrell, G. L., *ibid.*, 1952, v12, 968.

6. Farrell, G. L., and Richards, J. B., PROC. SOC. EXP. BIOL. AND MED., 1953, v83, 628.

Received May 18, 1954. P.S.E.B.M., 1954, v86.

Effects of Neonatal Anoxia on Maze Learning in Rats. (21092)

JOHN W. RICHARDSON.* (Introduced by Frank A. Beach.)

From the Department of Psychology, Yale University.

It is generally agreed that severe oxygen deprivation at the time of birth can result in a variety of behavior disorders involving intellectual deficit. These effects may be due solely and directly to neonatal anoxia(1,4, 6,8). An alternate possibility is that failure to breathe normally at birth is a secondary effect resulting from congenital neurological defects which are also responsible for the psychological abnormality often associated

* Present address: College of Physicians and Surgeons, Columbia University, New York City.

with early oxygen deprivation.

In an attempt to study the behavioral effects of neonatal anoxia in otherwise normal individuals a few experiments on animals have been conducted(3,5). Investigations of the learning ability of rats rendered partially anoxic shortly after birth have revealed some impairment, but statistically significant decrements have not been produced. In none of the earlier studies was oxygen deprivation complete, and in all instances the newborn animals had been breathing normally for some period of time before partial anoxia was induced. The present investigation was conducted to examine the long-term effects of anoxia induced before respiration had begun, to compare the effects of varying periods of total anoxia, and to measure these effects in a highly sensitive test.

Method. Induction of anoxia. Female rats were mated in pairs to fertile males so that both members of a pair would come to term at approximately the same time. At the expected time of parturition the animals were kept under constant observation and as soon as one female delivered her litter, the second animal was sacrificed by a sharp blow on the head and the abdomen was then opened to expose the uterus. Fetuses were left in the uterus for the desired period of anoxia after which they were removed and the membranes cleared away from the head so that respiration could begin. The anoxic period was taken to be the time elapsing between the cessation of maternal respiration and the initial inspiration shown by the experimental animal. As soon as respiration was established the young were placed with the female whose litter had just been born, and her own offspring were removed. This method of providing a recently parturient foster mother was necessary to insure a high rate of successful adoptions. Thirty-three experimental and 23 control animals were used. *Learning tests.* The Hebb-Williams closed field maze was used to measure learning(2). This method of testing learning ability has been standardized and refined by Rabinovitch and Rosvold who found it more reliable than other mazes and more sensitive in reflecting deficits produced by small lesions in the cerebral cortex(7). The maze consists of a plywood box 3 feet square with the entrance alley and food compartment at opposite corners. Movable barriers are arranged in various patterns to create 6 different practice problems and 12 different test problems. Areas lying outside of the most direct path from the starting alley to the food compartment are considered error zones, and an animal's score consists of the total number of entrances into error zones in the course of 8 runs to the food on each problem. One unique and valuable feature of this device lies in the use of a succession of different problems which permits generalization or transfer from one pattern to the next. It might be said to measure the rat's ability to "learn to learn."

Testing schedule. When the subjects were 58 days old adaptation to the maze was begun. Animals were deprived of food for 24 hours and were subsequently fed only in the food compartment of the maze. The duration of the feeding period was adjusted for each individual so as to maintain body weight approximately 15 g below the weight at satiation. Different practice problems were presented twice each day until adaptation was complete. The criterion for adaptation was 9 runs to the food tray in not over 60 seconds on 2 consecutive problems. This criterion had been met by the time the rats were 65 days old, and at this point the 12 learning tests were begun. Rats were tested on two different problems each day for 6 days. Daily sessions were spaced 10 hours apart. Every animal was allowed 8 runs through the maze on each of the 12 problems, and for each problem the total number of errors made in reaching the food compartment was recorded. The testing procedure was held as constant as possible and the order in which the animals were tested was varied for each problem in an attempt to control the possibility that rats run late in a series might follow odor trails left by animals run previously.

Results. General effects of anoxia. It was found that a large proportion of the rats could survive to adulthood after a 20-minute period of anoxia at birth, but none of the animals prevented from breathing for 21 minutes or longer could be revived even temporarily. Within the lethal limit there was no clear cut relation between mortality and the duration of anoxia. Fifty-nine percent of the control animals survived to the termination of the investigation at 71 days. Within the experimental group, survival to 71 days after 1-10 minutes of anoxia was 69%, after 11-15 minutes it was 86%, and after 16-20 minutes, 72%. The great majority of deaths occurred within the first 48 hours or so after birth. No relation was found between the duration of anoxia and the weight and general health of the animals throughout the course of the study. The 33 experimental animals were subdivided on the basis of the duration of anoxia as follows: 1-10 minutes, 16 animals; 11-15 minutes, 7 animals; 16-20 minutes, 10.

Maze performance. Control animals made an average total of 178.4 errors on the 12 tests combined, and the comparable score for the experimental group was 194.0. This difference was significant at the .06 level of confidence (t-test, one tail). Within the experimental group there was no consistent relationship between the duration of the anoxic period and the number of errors made in the maze. *Control* rats made an average of 94.2 errors during the first 6 tests and 84.2 errors in the last 6. This difference was significant at the .05 level. In contrast, the average number of errors for experimental animals was 97.3 in the first 6, and 96.7 in the last 6 tests. This difference was not statistically significant. Average error scores for control and experimental rats did not differ significantly during the first 6 tests, but control animals were significantly better than experimental rats in the last 6 tests ($p = .04$). Although the experimental group as a whole was inferior to the controls, there was no statistically significant relation between duration of the anoxic period and progressive improvement during the 12 tests. Absence of such a relationship may have been due to the small number of cases in some of the experimental groups.

Summary and conclusions. Periods of anoxia ranging from 1 to 20 minutes were induced in unborn rats by preventing the initiation of respiration before removing the full term fetuses from the uterus. Beginning at 65 days of age, the experimental and control animals were tested on the Hebb-Williams closed maze. In a series of 12 standardized tests the experimental rats made more errors than the controls ($p = .06$). The inferiority of anoxic animals was most marked in the final set of 6 tests during which they were significantly inferior to the control group ($p = .04$). Control rats displayed progressive improvement in performance in successive maze problems, but no such improvement was evident in the records of experimental cases. It appears that oxygen deprivation at the time of birth tends to reduce the rat's ability to transfer prior learning to new situations. Within the experimental group, however, there was no relationship between the duration of the anoxic period and the extent of deterioration in maze performance with respect to either total scores or improvement in successive tests.

1. Doll, E. A., Phelps, W. M., and Melcher, R. T., *Mental deficiency due to birth injuries,* New York, Macmillan, 1932, pp45, 17-18.

2. Hebb, D. O., and Williams, K. A., *J. genet. Psychol.,* 1946, v34, 59.

3. Hurder, W. O., and Sanders, A. F., *J. comp. physiol. Psychol.,* 1953, v46, 61.

4. Little, W. J., *Trans. Obst. Soc.* (London), 1861, v3, 293.

5. Muir, G. W., and Bunch, E. M., *J. comp. physiol. Psychol.,* 1950, v43, 436.

6. Preston, M. I., *J. Pediat.,* 1945, v26, 353.

7. Rabinovitch, M. S., and Rosvold, H. E., *Can. J. Psych.,* 1951, v5, 122.

8. Schreiber, F., *J.A.M.A.,* Ill., 1938, v1263.

Received May 19, 1954. P.S.E.B.M., 1954, v86.