16630

Relative Lack of Myelin in Optic Tracts as Result of Underfeeding in the Young Albino Rat.*

A. R. Buchanan and J. E. Roberts.

From the Department of Anatomy, University of Colorado Medical Center, Denver.

The underfeeding experiments reported herein were originally started, and are still being carried on, to determine the effects of inanition on the ability to regulate body-temperature. We have previously presented experimental evidence indicating that normal myelination of hypothalamic fibers of young rats may have an important bearing upon the attainment of regulatory ability.[1] The myelin lack in the optic tracts of underfed animals has been an incidental finding, but one which may have considerable practical importance.

Donaldson[2] studied the effects of underfeeding on myelination in the central nervous system of the albino rat. His rats were 30 days old at the beginning of the experiment and 51 days old when sacrificed. The average percentage difference in body weight, at the end of the experiment, between his control and underfed animals was 41.2, and the average percentage difference in brain weight was 7.7. Weigert-stained sections did not show grossly observable differences between the myelin content of the brains from the control and those from underfed rats. Donaldson concluded from these studies that underfeeding does not arrest myelination.

Methods and Materials. Six litters of Wistar strain albino rats have been used in the present investigation. On the 17th to 20th day of life, 3 rats from each litter were weaned, and placed in individual cages, where each was maintained on a diet of 5 g of Purina laboratory chow per day. The control animals were left with their mothers until they had reached 26 days of age, when they were weaned and then maintained on Purina laboratory chow *ad libitum*. The underfed animals and their litter-mate controls were sacrificed on the 50th to the 52nd day of life.

When sacrificed, the experimental and control animals were perfused with Regaud's solution (3½% potassium dichromate—80 parts; neutral formalin—20 parts). Following perfusion, the brains were removed, weighed, and placed in 5% potassium dichromate. They were left in this solution for 10 days at 38-40°C; the solution was renewed on the 2nd, 5th and 8th days. When all the brains from a given litter had been embedded in paraffin, they were sectioned at 20 micra and mounted on slides. Representative slides from all the animals in the litter were placed together in a staining rack and stained en masse according to the method reported by Ulett, Dow and Larsell.[3]

The concentration of myelin in the optic tract was determined on the basis of monochromatic light absorption by stained sections as previously described.[1] After focusing upon the optic tract the slide was moved to an adjacent area which was free of sections and, without altering this focal adjustment, the amount of light entering the microscope was adjusted by means of a rheostat and the iris diaphragm at the inlet of the monochromater to give a reading of 80.0 on the galvanometer of the electrophotometer. The light was adjusted to this same "zero" reading in relation to each of the determinations in a given series, and this compensated for differences in thickness of slides, cover slips, and mounting media. When the light had been so adjusted, the optic tract was moved back into the field and the galvanometer reading record-

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1 Buchanan, A. R., and Hill, R. M., Proc. Soc. Exp. Biol. and Med., 1947, **66**, 602.

2 Donaldson, H. H., *J. Comp. Neurol.*, 1911, **21**, 139.

3 Ulett, G., Dow, R. S., and Larsell, O., *J. Comp. Neurol.*, 1944, **80**, 1.

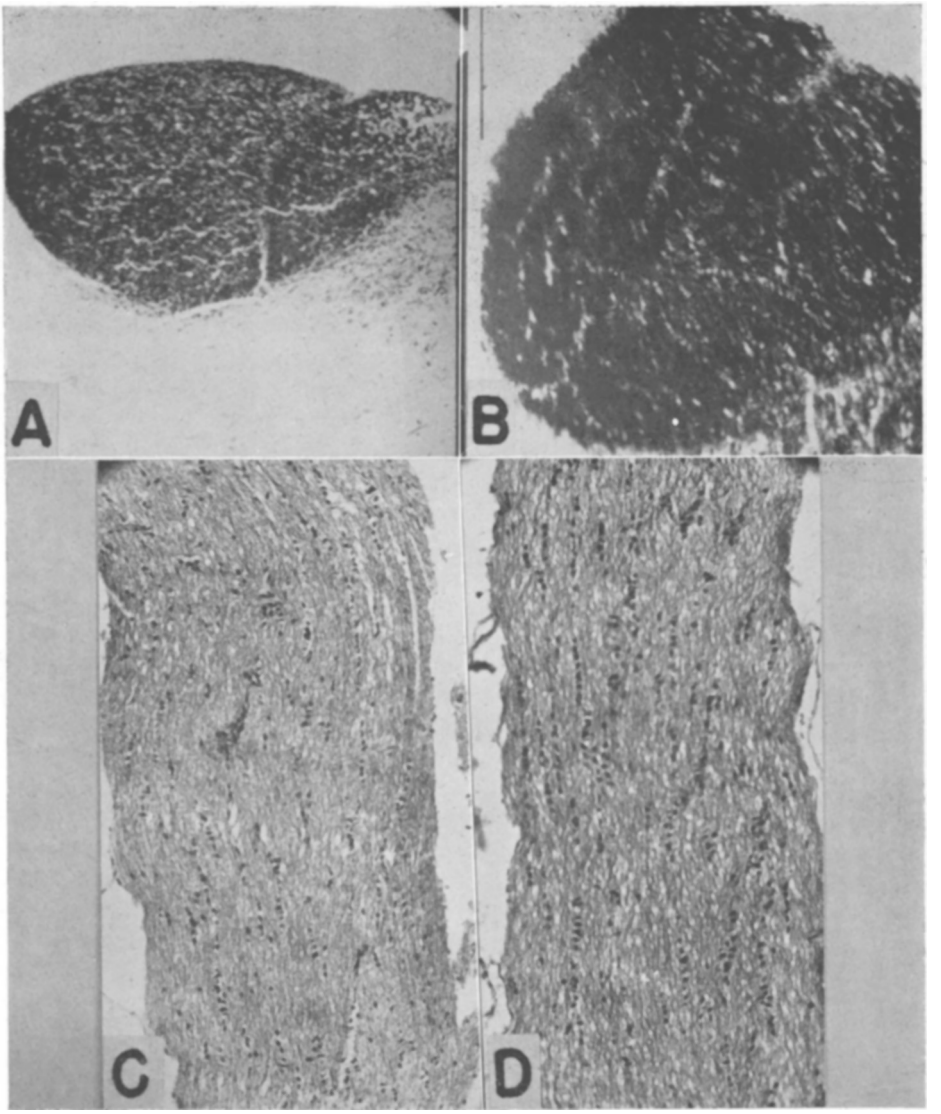

FIG. 1.
A. Optic tract from rat 1-F-3 (exp.); B. Optic tract from rat 1-F-4 (control); C. Optic nerve from rat 2-O-3 (exp.); D. Optic nerve from rat 2-O-6 (control). The optic tracts were stained for myelin by the method of Ulett *et al.*, and the optic nerves were stained by the method of Weil.

ed. The difference between this reading and the zero reading represented the amount of light absorbed by a given section. This procedure was carried out on 6 sections from a given animal. One hundred times optical density was computed for each reading as a measure of relative myelin concentration. The optical density here used was the log of the ratio of incident (galvanometer reading of 80.0) to ensuing light intensity in accordance with the Beer-Lambert law of absorption.

Results. A relative lack of myelin in the optic tracts of underfed rats, as compared to that present in those of litter-mate controls, appeared in the majority of cases. In many instances the difference was of sufficient magnitude to be apparent without the use of the electrophotometer. This is demonstrated by the photomicrographs presented in Fig. 1, in which the optic tracts from Rat 3 (ex-

TABLE I.
Summary and Statistical Evaluation of Results.

Litter	Myelin (experimental)	Myelin (control)	% difference	Significance	% difference body wt	% difference brain wt
Q	35.6	35.9				
	37.6	43.3	8.0	P = 1 (approx.)	28.6	13.5
	41.0	45.0				
S	29.9	41.7				
	38.2	43.6	12.6	P = 1 (approx.)	64.9	22.0
	28.6	25.4				
Y	42.6	50.2				
	35.1	55.2	19.8	P = 0.05	70.7	21.0
	45.3	47.9				
1-F	60.6	63.8				
	32.4	55.9	27.7	P = 0.15 (approx.)	57.6	14.9
	37.4	60.4				
2-K	39.8	46.4				
	31.3	40.5	15.9	P = 0.16 (approx.)	60.7	17.5
	33.3	37.2				
2-O	29.2	52.2				
	36.7	43.6	34.5	0.05 > P > .01	48.3	13.5
	27.0	46.2				
Means	(36.8)	(46.4)	19.8	P < 0.01	55.1	17.1

perimental) and Rat 4 (control) of litter 1-F are illustrated (A and B) together with the optic nerves from Rat 3 (experimental) and Rat 6 (control) of litter 2-0 (C and D). The optic nerves were not studied routinely since, in those cases in which they were, those from underfed rats showed the same relative lack of myelin as was apparent in the tracts of the same animals.

Gross examination of the optic foramina of the underfed animals has failed to show any evidence that the amyelination observed in their optic nerves and tracts is due to stenosis of the foramina such as has been described by Moore[4] in carotene-deficient calves.

Statistical analytical methods have been applied to our data by one of us (J.E.R.) and have served to show that the spectrophotometric method is reliable and that the results have statistical significance.

The average values of 6 determinations of relative myelin concentration (100 x optical density) for each underfed and control animal within the respective litters are shown in Table I. The percentage differences given in the table are based upon differences of means between underfed and control rats, relative to the mean of the controls. The succeeding column gives the approximate significance of this difference for each litter based upon analysis of variance within the respective litters. The last 2 columns give average per cent decrease in brain weight and body weight for each litter to indicate relative magnitude of inanition.

An analysis of variance was applied to the data of each litter to determine the sources of variation (1) between the myelin concentrations of underfed and control animals, (2) among the original determinations of myelin concentration, and (3) among the myelin concentrations of underfed and starved animals of each litter. The experimental errors (differences in section thickness, staining, spectrophotometric readings, etc.) were negligible when compared with the two other sources of variation in the experiment, since mean squares ranged from 2 to 7 in all litters except 2-K, where the value was 21. There was, in general, considerable variation between myelin concentrations among animals within

[4] Moore, Lane A., *J. Nutrition*, 1939, **17**, (Supp.), 10.

the control and experimental groups of each litter; mean squares ranged from 92 to 193 in all litters except 1-F, where the value was 725.

Comparison of the variance between underfed and control groups, where mean squares ranged from 98 to 2475, with the variance among animals within the groups becomes the critical factor of the analysis within litters. The F-test gave indications of significance as shown in the table. By this test the differences in myelin concentration could have occurred by chance within litters Q and S due to large variance among animals of experimental and control groups, are of moderate degree of significance in litters 1-F and 2-K, and are highly significant in litters Y and 2-O.

In determining the signifiance of underfeeding on optic tract myelination in all 6 litters, 2 major sources of variation, namely, differences in staining between litters and inherent differences in myelin content between litters, were not segregated experimentally. While such inherent variations between litters would be of considerable interest, it appears, from the standpoint of determining the effect of underfeeding on myelination, that the factor of greatest importance is the significance of the differences in myelin density that appeared in the entire group of 6 litters. An F-test comparing total variance between underfed and control animals of each litter with the total variance within litters gave high significance to the difference in myelin ($P < 0.01$). Thus, for the total experiment, there seems to be a significant difference of myelin concentration between control and underfed rats of the order of magnitude of 20%.

Discussion. The relative lack of myelin in the optic nerves and tracts of the majority of our underfed rats, determined photometrically, must be considered as indicative of partial or complete arrest of the process of myelination. Curves constructed from photometric determinations of myelin densities in the optic tracts of successively older members of several litters of albino rats indicate that myelination within the visual pathway continues to be an active process until sometime between the 40th and 50th days as compared with its completion in the medullary pyramid and hypothalamus between the 18th and 30th days.[1] This delay in myelination of the components of the visual pathway may be due to a lack of functional stimulation until after the eyes have opened (14th to 17th day). Tilney and Casamajor[5] reported that they were able to correlate beginning myelination of the optic nerves and the eye-opening reaction in kittens.

The accuracy of the results reported herein depends upon the assumption that the myelin concentration is directly related to optical density in accordance with the Beer-Lambert Law of Absorption. The use of monochromatic light seems to validate this assumption. Whereas slight deviation from the law may be present, the differences found for the entire group of 6 litters appear to be of sufficient magnitude and reliability that such deviation should not alter the general conclusions.

Summary and Conclusions. Quantitative limitation of food intake sufficient to account for average body-weight differences of 55% between underfed and litter-mate control rats, has also resulted in statistically significant differences in myelination of the optic tracts and nerves. The concentration of myelin in the optic tracts of the underfed rats has averaged 20% less than that in the control animals. Since myelination of the optic tracts, as shown by spectrophotometric determinations in litter-mate rats at successively older ages, is not complete until sometime between the 40th and 50th days, the conclusion seems justified that the relative lack of myelin observed in the underfed animals is due to partial or complete arrest of the process of myelination.

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5 Tilney, F., and Casamajor, L., *Arch. Neur. and Psychiat.*, 1924, **12**, 1.