ones. No studies of penicillinase production were carried out; in view of the results of Spink and Ferris with *Staphylococcus aureus,* similar investigations on susceptible and *in vitro*-produced resistant strains of *Streptococcus pyogenes* would be very interesting.

Although one strain of *Streptococcus pyogenes,* which was relatively resistant to penicillin, has been described[2] this organism was isolated from a patient before therapy with the antibiotic agent was instituted. There have been no reports of the development of decreased susceptibility to penicillin during treatment of patients with streptococcal (Group A) infections with this drug. Hartman and Weinstein,[7] in a study of over 100 strains of *Streptococcus pyogenes* isolated from cases of scarlet fever were unable to demonstrate any abnormal degree of resistance in the strains originally isolated or in those recovered from patients who had been treated with penicillin and in whom recurrence of *Streptococci* in the pharynx had occurred after therapy was stopped. It would appear from these results, therefore, that the development of resistance to penicillin by *Streptococcus pyogenes* may be easily accomplished *in vitro,* but occurs only rarely, if ever, in patients treated with this agent.

Conclusions. 1. The resistance of *Streptococcus pyogenes* to penicillin has been increased from 4 to 32 times by frequent subculture in media containing increasing amounts of the antibiotic agent.

2. The resistance to penicillin which develops *in vitro* is only temporary and can be abolished fairly rapidly by frequent subculture in drug-free media.

7 Hartman, T. L., and Weinstein, L., unpublished data.

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Temperature Regulation in Albino Rats Correlated with Determinations of Myelin Density in the Hypothalamus.*

A. R. BUCHANAN AND R. M. HILL.

From the Departments of Anatomy and Biochemistry, School of Medicine, University of Colorado, Denver, Colo.

Newborn albino rats are unable to regulate their internal temperatures. When placed in a cold environment their temperatures descend rapidly to that of the environment; a warmer than normal environment results in a marked increase in internal temperature. It has been found that the ability of the young rat to regulate its internal temperature when placed in a cold environment gradually improves from birth to the age of 18-30 days, after which time it usually maintains its body temperature at or very near the normal level.[1]

It has been conclusively demonstrated that the hypothalamus is essential to temperature regulation.[2] Considerable numbers of small myelinated fibers are consistently found in the lateral hypothalamus of the adult brain.[3] In spite of the diverse opinions which have appeared in the literature for many years regarding the correlation of function of neurons with myelination of their axons, it has been considered worthwhile to investigate myelination in the hypothalamus and to attempt correlation of the development of myelin with the beginning of the temperature regulating function of this portion of the brain.

The early literature concerning myelination

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1 Hill, R. M., *Am. J. Physiol.*, 1947, **149**, 650.

2 Ranson, S. W., *Res. Publ. Assn. Nerv. Ment. Dis.*, 1940, **20**, 342.

3 Ingram, W. R., *Res. Publ. Assn. Nerv. Ment. Dis.*, 1940, **20**, 195.

of nerve fibers and possible correlations between myelination and function has been reviewed by Tilney and Casamajor[4] who also studied somatic activity in the kitten and correlated their observations with the distribution of myelin sheaths in the different fiber paths. They concluded from their investigation that myelinogeny in connection with behavioral components affords strong evidence in the cat that the deposition of myelin is coincidental with the establishment of function in definite fiber systems. They conceded that this coincidence might not, however, be the case in white rats in which both Watson[5] and Donaldson[6] reported complete absence of myelin in the nervous system at birth.

Langworthy[7] studied decerebrate preparations of kittens, young rabbits, and young guinea pigs. He found that myelination in the lower portion of the brain stem and the spinal cord was incomplete in kittens and rabbits in which decerebration was followed by movements of prolonged progression. In more mature animals in which decerebration was followed by the development of typical extensor rigidity, myelination of the long tracts was far more complete. He considered that correlation between myelination of the rubrospinal tract and the occurrence of decerebrate rigidity was strongly indicated.

Langworthy[8] also studied the behavior of pouch-young opossums and attempted to correlate their behavior with myelination of tracts in the central nervous system. He wrote, "The evidence that tracts in the central nervous systems become medullated at the time when they become functional is certainly suggestive, but by no means positive. No one who studies the opossum can evade the fact that this animal is capable of complicated reflex activity before any myelinated fibers are present." Angulo,[9] on the basis of observations of discrete reflexes in rat fetuses, stated that myelination is not a criterion of "functional insulation." Windle, Fish, and O'Donnell,[10] studying cat fetuses of ages from 42 to 60 days after insemination, found a high degree of reflex activity to be present in the fetuses even though the tracts involved were still unmyelinated. They concluded that myelination is not correlated with function in the absolute sense, but conceded that conduction in a given pathway may be improved after myelin sheaths have been acquired.

Ulett, Dow, and Larsell[11] investigated the inception of conductivity in the corpus callosum and the cortico-ponto-cerebellar pathway in young rabbits. They found that myelination of the fiber systems studied is not essential for the conduction of nerve impulses produced by electrical stimulation, and that conduction of such impulses precedes myelination of the fibers by several days in the rabbit. They did, however, observe an increased capacity for conduction in the larger and older animals.

Methods and Materials. Records of the temperatures of rats subjected to an environmental temperature of 5° to 8°C have been obtained through the use of iron constantan and copper constantan thermocouples sealed within the tips of ureteral catheters. Such catheters are easily inserted through the anus and rectum into the colon of rats as young as 4 days of age. The e.m.f. developed by the thermocouple at varying internal temperatures of the animal was originally measured with a Leeds-Northrup potentiometer. Each thermocouple was carefully calibrated with reference to the potentiometer before being used for the recording of temperatures. More recently temperatures have been directly recorded by means of a Brown electronic recording potentiometer. All temperature determinations were made with the

[4] Tilney, F., and Casamajor, L., *Arch. Neur. and Psychiat.*, 1924, **12**, 1.

[5] Watson, J. B., *Animal Education, Chicago Univ. Contrib. to Phil.*, 1903, No. 4.

[6] Donaldson, H. H., *American Textbook of Physiol.*, Phila., 1901.

[7] Langworthy, O. R., *Carnegie Inst. of Washington, Contrib. to Embryol.*, 1926, No. 89, **17**, 125.

[8] Langworthy, O. R., *J. Comp. Neur.*, 1928, **46**, 201.

[9] Angulo, A. W., *J. Comp. Neur.*, 1929, **48**, 459.

[10] Windle, W. F., Fish, M. W., and O'Donnell, J. E., *J. Comp. Neur.*, 1934, **59**, 139.

[11] Ulett, G., Dow, R. S., and Larsell, O., *J. Comp. Neur.*, 1944, **80**, 1.

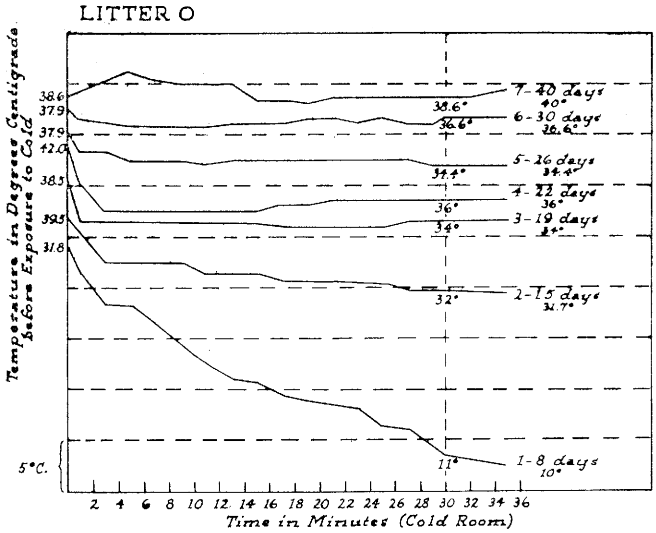

FIG. 1.
Temperature curves obtained from the members of one litter (Litter O) during 35 minutes in the cold room (5°-8°C). The ages (in days) of the respective animals are indicated.

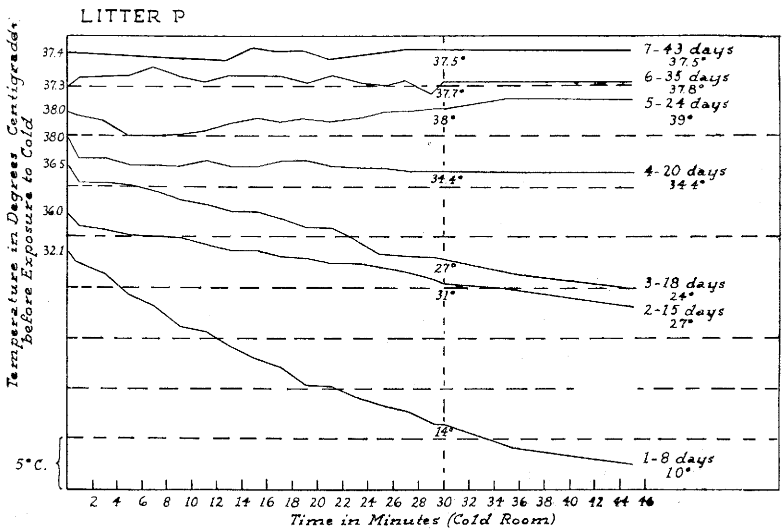

FIG. 2.
Temperature curves obtained from the members of Litter P during 45 minutes of exposure to cold (5°-8°C).

thermocouple inserted to the level of the diaphragm, thus assuring high colonic rather than rectal temperatures. It has been shown that such temperatures more accurately represent the thermal condition of the animal than those obtained by rectum, since the latter fluctuate with changes in the environmental temperature.[1]

The experimental procedure employed in our attempts to correlate temperature regulation with myelination of the hypothalamus has been carried out on twenty litters of Wistar strain rats. When a given litter had reached the age of 6 to 8 days, one animal from the litter was subjected to a cold environment (5-8°C) after first determining its body temperature in a room whose temperature was approximately 26.5°C. The temperature of the animal was recorded at two-minute intervals with the Leeds-Northrup potentiometer, or at one-minute intervals with the Brown electronic potentiometer. Temperatures were recorded during 30 to 45 minutes. In the preparation of Fig. 3, 4, and 5, the temperatures after 30 minutes in the cold room were used to illustrate graphically the difference between normal environmental temperatures and those resulting from exposure to cold. Following exposure to cold, the animal was anesthetized with ether and perfused with one of three fixatives—10% neutral formalin, Erlicki's solution (copper sulphate and potassium bichromate), or 3½% potassium bichromate. The first of these preceded the Weil stain for myelin,[12] the second is the fixative recommended by Kultschitzky for his myelin stain,[13] and the third is that found most satisfactory for the myelin stain used by Ulett and his collaborators.[11] After perfusion, the brain was removed, fixation was continued for the specified period, and in the solutions specified by the originator of the stain. A block from the center of the brain which included the hypothalamus, or a complete half brain was finally imbedded in paraffin, sectioned, and mounted on slides. Other animals from the same litter were similarly treated at appropriate intervals until the litter was exhausted. The same fixatives were applied in a uniform manner to the brains of all the members of a given litter. When all the hypothalami from a given litter had been sectioned at as uniform a thickness as is possible with the Spencer rotary microtome and placed on slides, staining of the sections was carried out. In order that comparisons based on the density of the myelin stain could be made between the hypothalami of the rats of different ages, three slides (9-12 sections) were selected from each animal in the litter. These were placed in a staining rack and stained en masse. Theoretically, if

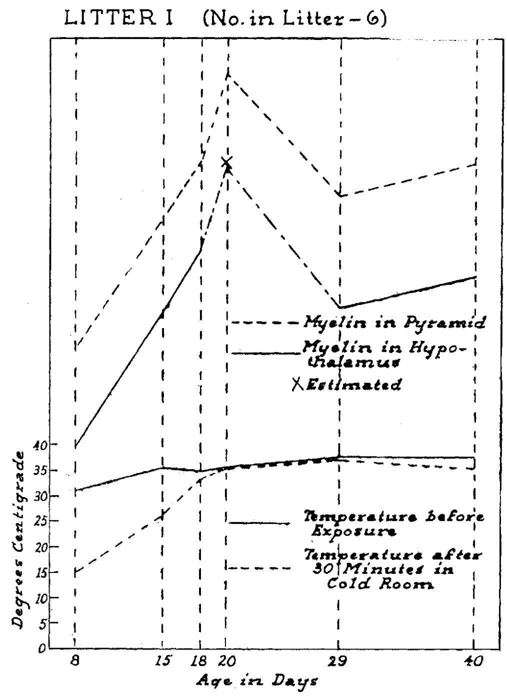

FIG. 3.

Records of temperatures before and after 30-minute exposures to cold and curves representing the density of myelin staining in the pyramid and lateral hypothalamus as determined photometrically. (Technical difficulties, resulting in loss of sections of the hypothalamus in the 20-day animal, made an accurate determination impossible.)

the stain is specific for myelin, those hypothalami containing the least myelin (or no myelin) should be less deeply stained than those in which myelination is further advanced. In our experience the myelin stain reported by Ulett, Dow and Larsell[11] has given the most satisfactory results, since the nerve cells remain almost entirely unstained in sections prepared according to their method.

Determinations of staining density have utilized a photometric method. The light from a Spencer microscope lamp has been directed through a monochromator to the substage of a binocular microscope; the monochromator was set to deliver light at a wavelength of 600 mu. The phototube of a Photovolt electronic photometer is firmly attached to the right ocular of the microscope in such a manner that all light traversing that ocular

[12] Weil, A., *Arch. Neur. and Psychiat.*, 1928, **20**, 392.

[13] Lee, B., *The Microtomist's Vade-Mecum*, 10th ed., p. 525.

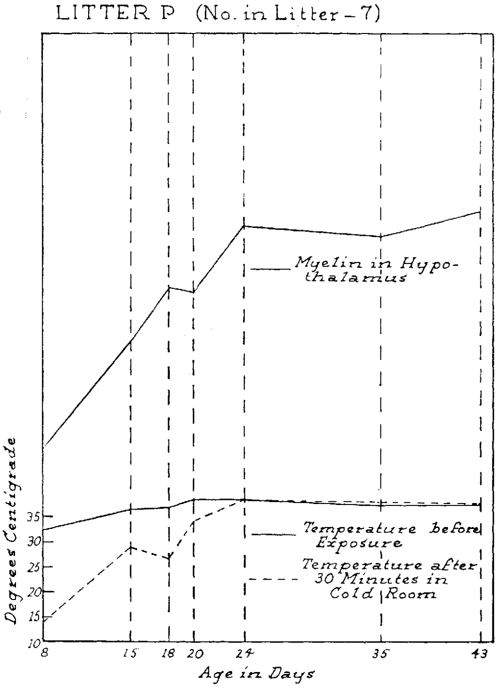

FIG. 4.

Records of temperatures before and after 30-minute exposures to cold and curve representing the density of myelin staining in the lateral hypothalamus in Litter P. (*Cf.* Fig. 2.)

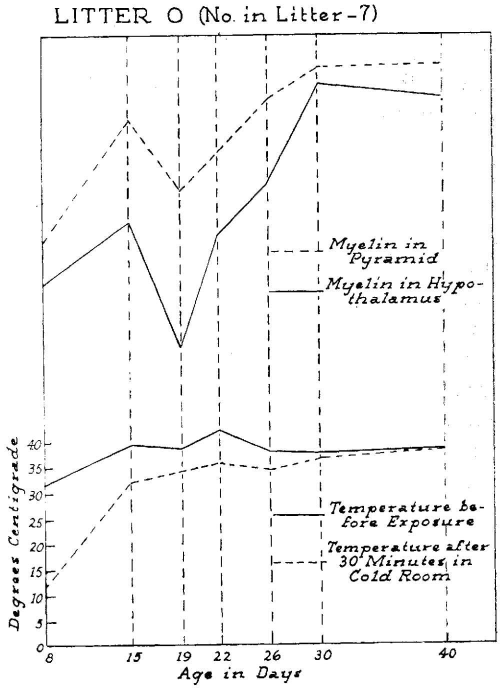

FIG. 5.

Records of temperatures before and after 30-minute exposures to cold and curves representing the density of myelin staining in the pyramid and lateral hypothalamus in Litter O. (*Cf.* Fig. 1.)

is focused upon the photoelectric cell and thus recorded directly upon the galvanometer of the instrument. The procedure for obtaining the density of the myelin stain in a given section consisted of focusing upon the lateral hypothalamic area through the left ocular of the microscope after which the slide was moved to an area free of sections; without altering the focal adjustment of the microscope, the left ocular was covered and the light entering the microscope was adjusted by means of a rheostat and the iris diaphragm at the inlet of the monochromator to give an arbitrary galvanometer reading, which "zero" reading was then kept constant for an entire series of determinations. The maintenance of a constant "zero" point on the scale compensated for differences in thickness of slides, cover slips, and mounting media. After the light had been adjusted, the section was moved back into the field, the left ocular was again covered, and the galvanometer reading recorded. The difference between this reading and the "zero" reading represented the amount of light absorbed by the stained section. This procedure was repeated on several sections of the hypothalamus of a given animal, and the several readings were then averaged. The averages were used in the construction of myelin curves for the litter being studied. Typical myelin curves obtained by this method in three of the litters investigated are shown in Fig. 3, 4, and 5. Myelin determinations have also been made on the pyramid of the medulla in several of the litters studied (Fig. 3 and 5) in order to check myelination in an area completely free of nerve cell bodies.

Results. The temperature curves during exposure to cold in animals from the same litter at progressively older ages are illustrated in Fig. 1 and 2. These are entirely consistent with previous obervations by one of us[1] and show a progressive improvement in ability to regulate against cold. Regulation, aside from an initial drop of some 3½ degrees, was fairly well established in animal 3 (Fig. 1) at 19 days of age, and was completely established in animals 6 and 7 at 30 and 40 days of age. Regulation in Litter P (Fig. 2) was quite good in animal 4 at 20 days of age, and was completely established in animal 5 at 24 days of age.

In the majority of litters studied, very adequate regulation against cold was evident at 18 and 20 days of age (Fig. 3). In some litters complete regulation appeared later (24 days in Litter P, Fig. 4, and at 30 days, Litter O, Fig. 5). The density of myelin stains as measured by the method described above has regularly reached a high level coincident with the beginning of regulation (Fig. 3, 4, and 5). In most of the litters studied, there has been a tendency for a levelling off or a depression of the myelin curve during the post-regulatory phase. As was to be expected, the pyramids of the medulla showed considerably greater degrees of staining density than the hypothalamus at all ages. There was, however, a striking parallelism between the curves representing density of the pyramids and of the hypothalamus in progressively older rats (Fig. 3 and 5).

The marked pre-regulatory depressions in the myelin curves for hypothalamus and pyramid in Litter O (Fig. 5) may have been due to a dietary deficiency, since we were unable to obtain lettuce for our rat colony at the time when this particular litter was born. The same dietary deficiency may have been responsible for the delay in attainment of regulatory ability and the corresponding delay in myelin formation as indicated by the fact that the curves reached their highest level in the 30-day animal.

Discussion. Koch and Koch[14] studied the chemical differentiation of the brain of the albino rat during growth. Their analyses were carried out on whole brains of rats 1, 10, 20, 40, and 120 days of age. The results of their investigation so far as phosphatides are concerned may be summarized as follows:

Age of rats in days	1	10	20	40	120
Phosphatides	15.2%	12.3%	21.4%	21.8%	21.6%

The phosphatides have been selected from their table as being the most likely substances having affinity for the various modifications of the Weigert stain for myelin which have been used in our experiments. The rather marked increase in phosphatides during the first 20 days compares favorably with the curves which have been obtained by our method of determining the concentration of myelin in the hypothalamus and pyramid of the albino rat. None of our investigations of myelin have been carried out on rats younger than 6 days of age, and we therefore have no material available for comparison with the figure of 15.2% phosphatides found in rats one day of age. It will be noted that Koch and Koch actually found a lower percentage of phosphatides in the brains of 10-day rats than in those one day old. The tendency for our myelin curves to flatten out or to be depressed during the period between the 20th and 40th days of life may possibly be due to the failure of phosphatides to increase appreciably during that period. Further studies are in progress to determine, if possible, the individual specificities of our myelin stains for lecithin and cephalin. Cholesterol will also be studied in this connection. Langworthy[15] called attention to the well known fact that animals, particularly those born in large litters, vary greatly in their maturity at birth and in the rapidity of their growth thereafter. We have studied litters of albino rats containing as many as 12 individuals, and have found that the attainment of the ability to regulate internal temperature when exposed to a cold environment is somewhat delayed in such large litters and that there is a tendency for the myelin curves to be much less steep in the period between 6 and 20 days than is the case in the smaller litters.

Watson[5] stated that myelination does not begin in the higher centers of the brain until rats have attained the age of 24 days. Although this may be true of cortical association fibers, it certainly does not appear to be true of the hypothalamus, if our method is valid for the determination of the presence of myelin.

[14] Koch, W., and Koch, M. L., *J. Biol. Chem.*, 1913, **15**, 423.

[15] Langworthy, O. R., *Carnegie Inst. of Washington, Contrib. to Embryol.*, 1929, No. **114**, **20**, 127.

The somewhat earlier development of resistance to cold in these animals (Wistar strain) as compared to those previously studied (Denver University strain) may be due to the difference in strain, or it may be due to the fact that in the earlier experiments lettuce was not a part of the diet of the mothers whereas it was in these experiments in all cases except Litter O (Fig. 5).

Conclusions. The optical densities of sections of the hypothalamus stained by modifications of the Weigert method have been measured in rats varying in age from 6 to 70 days by means of monochromatic light and an electronic photometer. These measurements have been correlated with the abilities of the animals to regulate their internal temperatures when exposed to a cold environment. We believe that our method has recorded densities which are dependent mainly upon the amount and/or character of the myelin in the hypothalamus at the respective ages studied, and that the increase in myelin from birth to 20 days of age may contribute to the acquisition of the capacity for temperature regulation. We do not believe that myelination in the hypothalamus is the only factor concerned in the acquisition of this function. Many other factors are no doubt operative and many of these are currently being investigated.

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Teratogenetic and Lethal Effects of Influenza-A and Mumps Viruses on Early Chick Embryos.

VIKTOR HAMBURGER* AND KARL HABEL.†

From the Industrial Hygiene Research Laboratory and the Division of Infectious Diseases, National Institute of Health, Bethesda, Md.,‡ and the Department of Zoology, Washington University, St. Louis, Mo.

Gregg[1] demonstrated a high incidence of congenital malformations in children whose mothers had contracted rubella (German measles) during the early months of pregnancy. These observations were confirmed by Swan[2,3,4] in Australia, and by American and European workers. Over 400 cases are now on record. The most common abnormalities caused by rubella are: cataract in one or both eyes, heart defects, microcephaly and deaf-mutism. The incidence of malformations following infection of the mother within the first 2 months of pregnancy is almost 100%, but low after the 4th month. It seems that a very early infection (around 1-1½ months of pregnancy) causes predominantly cataract, whereas a later infection (around the 2nd month) causes predominantly deaf-mutism.

The discovery that the rubella virus passes through the placenta and causes a specific pattern of localized malformations in the human embryo is of great interest from the point of view of embryology, virus biology and clinical obstetrics. It seemed to be of importance to analyze the effects of viruses as teratogenetic agents under the controllable conditions of animal experiments.

The chick embryo suggested itself as a suitable embryonic material, since it is known

* Special Research Fellow of the National Institute of Health.

† Surgeon, National Institute of Health.

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1 Gregg, N. McA., *Trans. Ophthalmological Soc. of Australia*, 1941, **3**, 35.

2 Swan, C., Tostevin, A. L., Moore, B., Mayo, H., and Barham Black, G. H., *Med. J. Australia*, 1943, **2**, 201.

3 Swan, C., Tostevin, A. L., Mayo, H., and Barham Black, G. H., *Med. J. Australia*, 1944, **1**, 409.

4 Swan, C., and Tostevin, A. L., *Med. J. Australia*, 1946, **1**, 645.