early in starvation and that the eventual content reflects minimal production.

The mechanism controlling production and release of gonadotrophic hormone during starvation has not been elucidated. The possibility that the amount of circulating gonadotrophins or estrogens are major factors in controlling pituitary content during starvation seems unlikely since gonadotrophin content remains approximately the same despite wide variations in the amount of circulating estrogens and gonadotrophins (high in gonadotrophin injected starved rats, low in starved rats and normal in normal rats).

As a general proposition, release, circulating amount and excretion of gonadotrophins tend to parallel each other. For example, following castration, pituitary content, amount in the circulation and amount in the urine are decidedly greater than for intact animals for all species tested. Following administration of large (unphysiological) amounts of steroid sex hormones to either castrates or normal animals, pituitary content, amount in the circulation and urinary excretion of gonadotrophins fall below normal. Investigation of the 13-lined ground squirrel has revealed that during the period of sexual inactivity (hibernation) gonadal atrophy is associated with decreased amounts of gonadotrophic hormones in the pituitary and in the circulating blood. Just preceding and during the period of sexual activity, there are increased amounts of gonadotrophins in the hypophysis and circulation.[11]

Exceptions to this general proposition, other than the dichotomy found in starvation, have been demonstrated. Lauson, Golden and Sevringhaus[12] observed that immature female albino rats just prior to the onset of puberty had high pituitary levels of gonadotrophins before more than negligible amounts appeared in the circulation. At puberty, release was affected, and with diminishing content, increasing circulating amounts were noted.

Summary. During starvation circulating gonadotrophins fall precipitously whereas the content of hypophyseal gonadotrophin remains as high as under normal circumstances. This is in direct contrast to the usual situation in which pituitary gonadotrophin content accurately reflects the amount released into the circulation.

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12 Lauson, H. D., Golden, J. B., and Sevringhaus, E. L., *Am. J. Physiol.*, 1939, **125**, 396.

16170 *

In vitro Development of Temporary Penicillin Resistance in Streptococcus Pyogenes.

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The treatment of bacterial infections with penicillin rarely leads to an increase in the resistance of the causative organisms to the antibiotic agent during the course of therapy. While such a phenomenon has been described for *Staph. aureus* and *Strep. viridans*, there is no substantiated evidence that it occurs with *Strep. pyogenes, D. pneumoniae, N. gonorrheae* or other organisms *in vivo*. *In vitro* studies, however, have shown that bacteria may be made less sensitive to penicillin by certain cultural manipulations. Thus, strains of *Staph. aureus*, which were originally susceptible to relatively small doses of penicillin, have been made quite resistant by repeated

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subculture in media containing increasing quantities of this agent. A review of the literature on this subject is not included in this paper; for this, the reader is referred to the recent article of Spink and Ferris.[1]

The development of two kinds of resistance to penicillin in *Staph. aureus* has been demonstrated by Spink and Ferris.[1] The first type is an adaptation that can be reproduced *in vitro* by culturing in media containing increasing quantities of penicillin. The resistance is only temporary, can be abolished by growing the organisms in antibiotic-free broth, and cannot be associated with the production of penicillinase. The second, which occurs in patients as a result of treatment with penicillin, gives rise to strains of *Staph. aureus* in which the resistance is permanent and cannot be decreased by cultural manipulation; these organisms produce penicillinase.

Reports of penicillin resistance in *Strep. pyogenes* are few. One strain of this organism which appeared to be naturally resistant to penicillin (sensitive to 0.625 units per cc) was isolated by Hirsch *et al.*[2] from a patient with scarlet fever. Resistance was present when therapy with the antibiotic agent was first instituted and did not develop as a result of treatment. *Strep. pyogenes* was made resistant to penicillin *in vitro* by McKee and Houck.[3] They decreased the sensitivity of 3 strains of *Staph. aureus*, one strain each of types I, II, and III of *D. pneumoniae* and one strain of *Strep. pyogenes* to penicillin by growing the organisms in broth containing increasing amounts of this drug. The resistance of the beta hemolytic *Streptococcus* was increased 30-fold in a period of 3 months; there was an accompanying loss of virulence. Thirty-two rapid transfers in plain broth or 2 months' storage in the icebox produced no alteration in the degree of acquired resistance. Reduced velocity of growth and variation in colonial form were observed during cultivation in media containing increasing amounts of antibiotic, but the organisms grew luxuriantly after transfer to fresh broth or to blood agar plates which contained no drug. No change in type of enzyme activity was apparent although fermentation reactions were much delayed.

The purpose of the present paper is to report the development of resistance in several strains of *Streptococcus pyogenes* following rapid subculture in broth containing increasing amounts of penicillin and to demonstrate the temporary nature of this resistance as shown by its abolishment by rapid transfer in broth containing no drug. The mechanism of the development of penicillin resistance in bacteria is controversial and will not be discussed here. This subject is reviewed in detail in the papers of Luria,[4] Demerec,[5] and Spink and Ferris.[1]

Methods. Fifteen strains of *Streptococcus pyogenes* recently isolated from the pharynges of patients with scarlet fever were the organisms used in this study. All of the cultures were kept on blood-heart infusion-yeast-tryptose agar and transferred once a week, being kept in the icebox in the interim. Penicillin resistance was determined by the method of Rammelkamp.[6] Solutions of penicillin for incorporation into media were made up freshly each week and kept in the icebox when not in use. The medium in which penicillin resistance was produced consisted of double strength heart infusion-yeast-tryptose broth (0.5 cc) to which was added 0.5 cc of the desired dilution of penicillin and 0.1 cc of a 24-hour culture of *Streptococcus pyogenes*.

All the strains of *Streptococcus* were grown first for 24 hours in broth containing no drug and their resistance to penicillin determined, using an inoculum of between 10,000 and 100,000 organisms per cc. One-tenth cc of a 24-hour broth culture of each was then

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[3] McKee, C. M., and Houck, C. L., PROC. SOC. EXP. BIOL. AND MED., 1943, **53**, 33.

[4] Luria, S. E., PROC. SOC. EXP. BIOL. AND MED., 1946, **61**, 46.

[5] Demerec, M., *Proc. Nat. Acad. Sci.*, 1945, **31**, 16.

[6] Rammelkamp, C. H., PROC. SOC. EXP. BIOL. AND MED., 1942, **51**, 95.

inoculated into a tube of broth to which had been added the lowest concentration of penicillin which was inhibitory, as well as into 2 other tubes, one of which contained 20% and the other 40% more drug than the lowest dilution in which the strain grew. The cultures were incubated at 37°C for 24 hours and 0.1 cc of the one showing growth with the largest amount of penicillin was subcultured to 2 other tubes, one of which contained the amount of penicillin which previously had just allowed growth to occur and the other, 120% of this quantity of the drug. This procedure of culturing in the highest concentration of penicillin allowing multiplication of the organisms and in 120% of this amount was repeated every 24 hours except for weekends, when the transfers were made every 48 hours. All cultures showing growth were plated on blood agar every 3 days in order to check the purity of the strains and to determine any changes in colonial or cellular morphology.

After 41 transfers in penicillin-containing broth, 6 strains, the 3 with the highest and the 3 with only moderate increase in resistance to penicillin, were subcultured 16 times more in an attempt to increase to a greater degree their ability to withstand the antibiotic agent.

Following demonstration of penicillin resistance in these 6 strains of *Streptococcus pyogenes* (after 57 transfers), the organisms were cultured in broth containing no drug. Transfers were made every 24 hours and susceptibility to penicillin determined after 10 and 37 subcultures.

Results. The results indicating the development of resistance to penicillin by the strains of *Streptococcus pyogenes* studied are presented in Table I. The data demonstrating loss of resistance to the antibiotic agent following frequent subculture in drug-free broth are shown in Table II.

Determination of penicillin resistance after 41 subcultures in blood-heart infusion broth containing increasing amounts of penicillin revealed 3 strains with no appreciable alteration in their susceptibility to the drug, although they were approximately 2 times more resistant to penicillin than at the beginning of the experiment. This degree of change is within the limit of experimental error and cannot be considered significant. The resistance to penicillin of 4 strains (S-3, S-106, S-46 and S-47) increased 4-fold and of 5 strains (S-1, S-4, S-5, S-7, J-s) 8-fold after 41 transfers in penicillin-containing broth. One strain (S-9) was inhibited only by 16 times and 2 others (S-6, S-48), by 32 times the amount of antibiotic agent which stopped their growth at the start of the study.

Sixteen additional subcultures of 6 of the strains, a total of 57 transfers in drug-containing medium, revealed no further significant increase in penicillin resistance. One strain (S-47) revealed a 2-fold increase but this is within the limit of experimental error and, therefore, probably not significant.

Studies of the colonial and cellular morphology of the organisms at varying times during repeated transfer in penicillin-containing broth revealed no important changes. All of the strains produced matt colonies before and after being made drug-resistant. The colonies of the penicillin-insensitive variants were somewhat smaller than those of the drug-susceptible strains, although they varied considerably in size. Growth in broth and on blood agar plates was less rapid after frequent transfer in penicillin-containing media. No remarkable change in the ability of the *Streptococci* to produce hemolysin after developing resistance to the antibiotic agent was noted. The cellular morphology of the strains, as revealed by the Gram stain, underwent no alteration during the period of decrease in susceptibility to penicillin.

In order to determine whether or not the penicillin resistance which had been produced was temporary or permanent, 6 of the strains which had become relatively insensitive to drug were subjected to rapid subculture (every 24 hours) in broth containing no antibiotic agent. Determination of sensitivity to penicillin was carried out after 10 subcultures and revealed no significant change except with one strain which lost 50% of its resistance; this degree of change is, however, within the limits of experimental error. A

TABLE I.
Development of Resistance to Penicillin by *Streptococcus pyogenes* Following Rapid Transfer in Broth Containing Increasing Amounts of Penicillin.

Strain	Original resistance to penicillin, units/cc	Penicillin resistance after 41 transfers in broth containing penicillin, units/cc	No. of times more resistant to penicillin than original strain	Penicillin resistance after 57 transfers in broth containing penicillin, units/cc	No. of times more resistant to penicillin than original strain
S-1	.0075	.06	8x	—	—
3	.015	.06	4	—	—
4	.0075	.06	8	—	—
5	.015	.125	8	.125	8x
6	.015	.50	32	.50	32
7	.015	.125	8	.125	8
9	.015	.25	16	.25	16
10	.03	.06	2	—	—
106	.015	.06	4	—	—
J-s	.0075	.06	8	—	—
S-46	.015	.06	4	—	—
47	.015	.06	4	.125	8
48	.0075	.25	32	.25	32
49	.015	.03	2	—	—
50	.015	.03	2	—	—

TABLE II.
Loss of Penicillin Resistance of *Streptococcus pyogenes* After Frequent Subculture in Plain Broth.

Strain	Original resistance to penicillin, units/cc	Resistance to penicillin after 57 subcultures in broth containing penicillin, units/cc	Resistance to penicillin after 10 subcultures in plain broth, units/cc	Resistance to penicillin after 37 subcultures in plain broth, units/cc
S-5	.015	.125	.06	.015
6	.015	.50	.50	.015
7	.015	.125	.125	.0075
9	.015	.25	.125	.0075
47	.015	.125	.125	.015
48	.0075	.25	.125	.0075

striking increase in sensitivity to penicillin was present, however, after 37 subcultures in drug-free blood broth; all of the organisms returned to their original degree of susceptibility to the antibiotic. In the transformation from a drug-resistant to a susceptible state there was no change in the colonial or cellular morphology of the bacteria. The penicillin-sensitive streptococci appeared, however, to multiply somewhat more rapidly and to be more hemolytic than the resistant ones.

Summary and Discussion. The resistance of 12 out of 15 strains of *Streptococcus pyogenes* to penicillin was increased by daily subculture (41-57 times) in broth containing increasing amounts of penicillin, the maximum effect being obtained after 41 transfers; no significant increase was noted after 16 additional subcultures. The decrease in susceptibility to the antibiotic agent of 6 of the strains was temporary and was abolished rapidly by culture in drug-free media. No striking effects on the colonial and cellular morphology, degree of hemolysis, or rate of growth could be demonstrated during the process of acquisition or loss of sensitivity to penicillin. The drug-resistant strains, however, grew somewhat more slowly, produced slightly smaller colonies, and were a little less hemolytic than the highly susceptible

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.

16171

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

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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

* Supported by a grant from the Office of Naval Research.

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