random discharges were indicated in the VM, but a more or less systematic pattern of discharges was indicated in the LH.

Termination of ether inhalation increased the frequency of spontaneous unit discharges in the VM and decreased it in the LH, completely reversing both discharge patterns in all instances; that is, in the VM, the discharge interval no longer fitted the exponential distribution but the discharge frequency corresponded to the Gaussian one ($p < .05$ to .01), and in the LH, the discharge interval and frequency corresponded to the exponential and Poisson distributions, respectively ($p < .05$ to .01). This may explain the encephalographic patterns in unanesthetized cats where low-voltage, fast waves usually appear in the VM and slower ones in the LH (*7*). The changes in spontaneous unit discharge patterns may indicate some synaptic control which is influenced by anesthesia not only in the VM and LH, but also in other places such as the amygdaloid nucleus.

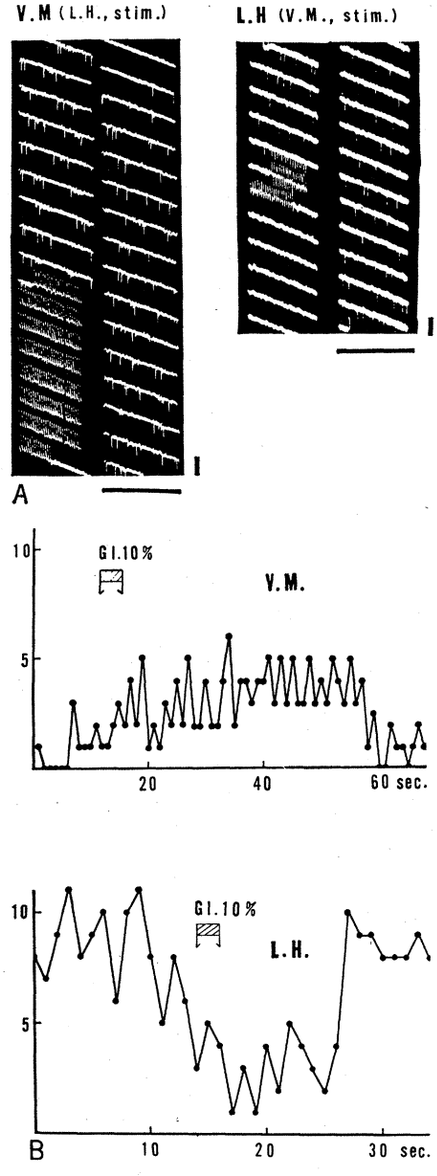

Fig. 2. Effect of electrical stimulation. *A*, From top to bottom and from left to right, (left) spontaneous unit discharges in the VM were reduced in frequency and even ceased for a few seconds by tetanic stimulation of the LH, (shown by artifacts in middle of left columns); (right) discharges in the LH were also decreased by stimulation of the VM. Two different preparations; time, 1 second. *B*, Effect of glucose solution. Number of discharges per second (ordinate) in the VM (top) and LH (bottom) were changed in frequency by injection of 0.3 ml of 10 percent glucose at the time shown. Abscissa, time.

With tetanic stimulation of the LH, discharges in the VM (21 of 25 neurons) decreased or even ceased, while discharges in the LH (15 of 16 neurons) increased up to about twice the former frequency during stimulation and even several seconds afterward, gradually returning to the original frequency (Fig. 2 *A*). The frequency of discharges increased up to about 200 percent in the VM and decreased in the LH when the intensity of stimulation was increased, probably because the stimulating current spread into the VM. Thus, the VM depression was not due to the spreading depression. With stimulation of the VM, the frequency in the LH decreased or ceased and then recovered (9 out of 10 neurons) in a manner similar to that when the LH was stimulated (Fig. 2 *A*). These results also indicate reciprocal relations between the VM and LH.

In 9 VM neurons of 13 and in 2 LH neurons of 30, the frequency of spontaneous unit discharges increased up to about 200 percent during or after injection of glucose solution. This effect sometimes lasted more than 30 seconds (Fig. 2 *B*). Glucose solution decreased the frequency to about half in 16 out of 30 neurons in the LH (12 were uneffected), but the original frequency was recovered several seconds after the injection (Fig. 2 *B*). On the other hand, injection of water accelerated the discharge in 12 out of 13 LH neurons. Sodium chloride solution increased the frequency in the LH (6 of 9 neurons), indicating that the glucose effects were not due to osmotic effect (except in 2 LH neurons). Tyrode's solution, used in control experiments, had no effect, and spontaneous unit discharges recorded from incorrect insertions were not influenced at all by any of the above treatments. This evidence seems to support the concept that glucose sensitive neurons are present in the ventromedial hypothalamic nucleus (*8*).

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Pteridines as Pigments in Amphibians

Abstract. *Extracts of brightly colored skins from nine amphibian species were analyzed chromatographically. In yellow skin in which xanthophores predominated, relatively large quantities of sepiapterin were found, while in red skin which was laden with erythrophores, three drosopterins were most prevalent. Frozen sections of skin indicated that pteridines were present within chromatophores, either alone or accompanied by carotenoids. It is concluded that sepiapterin and three drosopterins are utilized as pigments in amphibians and it is suggested that other less brightly colored pteridines also function in this respect. It no longer seems proper to make the tacit assumption that bright pigmentation of amphibians is due only to the presence of carotenoids.*

The ubiquity of integumental carotenoids among amphibians, together with the occurrence of yellow, orange, or red chromatophores which lose their color after treatment with fat solvents, has led to the general assumption that bright coloration of amphibians results from the presence of carotenoids in

Table 1. Principal chromatophore and pteridine found in analysis of pigmentation in adult skin of several amphibians. S, sepiapterin; D, drosopterins; Erythr., erythrophore; Xanth., Xanthophore.

Location	Color	Chromatophore	Pteridine	Solubility of pigment		Carotenoid
				Water	Fat solvents	
	Hemidactylium scutatum					
Dorsal	Cocoa brown		S			
	Plethodon cinereus					
Dorsal	Metallic red	Erythr.	D	+	−	−
	Eurycea lucifuga					
Dorsal	Dull red	Erythr.	D	+	−	−
	Eurycea bislineata					
Dorsal	Metallic yellow	Xanth.	S	+	−	−
	Pseudotriton ruber					
Dorsal and flank	Brick red	Erythr.	D	+	−	−
	Ambystoma maculatum					
Dorsal yellow spot	Bright yellow	Xanth.	S	+	−	−
	Bufo punctatus (young adults)					
Dorsal red spot	Bright red	Erythr.	D	+	−	−
	Hyla arenicolor					
Ventral and flank	Bright yellow	Xanth.	S	*	*	+
	Rana sylvatica					
Ventral	Yellow-red	Xanth.	S	*	*	

* Some in water and some in fat solvents.

cells known as xanthophores, erythrophores, or lipophores (*1*, *2*). Significant quantities of pteridines can often be extracted from yellow skin of adult frogs and salamanders (*3*, *4*). Moreover, three red pteridines (drosopterin, isodrosopterin, and neodrosopterin) and a bright yellow pteridine (sepiapterin) are present in skin areas of corresponding color of several cold-blooded vertebrates including the fishes, *Xiphophorus helleri* and *Platypoecilus maculatus* (*5*); the amphibians, *Rana temporaria* (*6*) and *R. japonica* (*4*) and four species of the reptile, *Anolis* (*7*). These recent observations together with older reports such as that of Ballowitz (*8*) concerning alcohol-resistant red chromatophores in amphibians, suggest that perhaps some bright coloration of amphibians is due to pteridines as well as to carotenoids. In order to understand more fully the participation of these brightly colored pteridines in pigmentation, the skins of several amphibian species which are laden with xanthophores or erythrophores were analyzed for the presence of pteridines, especially sepiapterin and the three drosopterins.

For the identification of pteridines, ascending paper chromatography was used on skin extracts (*9*) and on small pieces of skin squashed directly on paper (*10*). Solvents were mixtures of *n*-propanol and 1 percent ammonia (2:1); isopropanol and 2 percent ammonium acetate (1:1); *n*-butanol, acetic acid, and water (4:1:1); *n*-propanol, ethyl acetate, and water (7:1:2); and water-saturated butanol. Purified sepiapterin from *Drosophila melanogaster* (sepia strain) (*11*) and heads of *D. melanogaster* (wild strain) were used as controls to confirm the identity of red and yellow spots on chromatograms. Histological observations of skin were made on freshly frozen sections and on fixed materials. Localization of pteridines and carotenoids in sections utilized the differential solubilities of these compounds in fat solvents and in water. A water-soluble pigment which could not be removed by fat solvents (alcohol, chloroform, benzene, xylene, and ether) was assumed to indicate the presence of pteridine material and the absence of carotenoids. In some sections the presence or absence of carotenoids was determined by the sulfuric acid test (*2*). In this test carotenoids are revealed in freshly frozen sections by the appearance of a blue compound formed upon the addition of concentrated sulfuric acid.

Most of the results are depicted in Table 1, which records the pigmentary characteristics of adult skin from several amphibian species. Whenever xanthophores occurred, sepiapterin was present in relatively large amounts. There was a similar correspondence between erythrophores and all three drosopterins. Apparently, these pteridines are located within chromatophores for, in every case, either some or all of both the xanthophore and erythrophore pigments were water soluble. In *Plethodon, Eurycea, Pseudotriton, Ambystoma*, and *Bufo*, none of the red or yellow pigments appeared to be fat soluble; moreover, the sulfuric acid test indicated an absence of carotenoids. In *Hyla arenicolor* and *Rana sylvatica*, some of the yellow pigment could be removed by fat solvents and in *H. arenicolor* a positive sulfuric acid test was revealed. It appears, therefore, that in the first seven species listed, bright coloration is imparted exclusively by sepiapterin and the three drosopterins and in the last two species, yellow coloration is due to the presence of both pteridine and fat-soluble pigments, undoubtedly carotenoids. It is interesting that generally, whenever sepiapterin is the predominant pteridine, a small quantity of drosopterins is found and similarly, a prevalence of drosopterin is accompanied by a minimum of sepiapterin. Possibly these pteridine types are utilized together to yield a blend of red and yellow, or perhaps their simultaneous occurrence may be the fortuitous result of closely related synthetic pathways.

Drosopterins and sepiapterin are by no means the only pteridines found in the skin of the amphibians mentioned. Other pteridines, including isoxanthopterin, biopterin, and ranachrome-3 (*12*), are present in relatively small amounts. The fact that these compounds are not brightly colored, together with their presence in low concentrations, appears to preclude their use as pigments. However, in some anuran species (*Rana catesbeiana, R. pipiens*, and *Hyla cinerea*), large amounts of isoxanthopterin, biopterin, and ranachrome-3 are found associated with yellow pigmentation while sepiapterin is lacking. Moreover, xanthophores in these areas contain a water-soluble yellow pigment. Thus it seems reasonable that in some way even these pale pteridines can operate as pigments, if not in the free form, perhaps in conjugation with other substances or as polymers.

The results indicate that in some amphibians the pteridines, drosopterin, isodrosopterin, neodrosopterin, and sepiapterin function as pigments in erythrophores and xanthophores. With

respect to numbers of species, the distribution of pteridines is unknown. However, it seems quite certain that as more species are studied, more pteridines occurring as pigments will be revealed. It is no longer proper, therefore, to make the tacit assumption that bright pigmentation of amphibians is due only to presence of carotenoids. Actually, from preliminary results in our laboratory, there is still another class of compounds, the flavins, that contribute to bright pigmentation.

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Effect of Cold on the Presence of Staphylococcus aureus in the External Nares of the Rat

Abstract. *There is an increase in the recovery from the external nares of rats exposed to cold of colonies of* Staphylococcus aureus *that ferment mannitol and produce coagulase.*

Many factors influence the establishment and maintenance of the carrier state of *Staphylococcus aureus*. The influence of external environment is one of these factors. However, conflicting reports exist in the literature concerning its importance in altering the relation between host and parasite. Mayyasi, Birkeland, and Dodd (*1*) report that the normal bacterial flora of the nasal cavity of mice is markedly reduced by low humidity. Marcus, Miya, Phelps, and Spencer (*2*) note an effect of acute and chronic low-temperature stress on the survival of mice challenged with *S. aureus*. Furthermore, Dooley and Davis (*3*) state that the external environmental factors of ambient temperature and humidity influence the staphylococcal nasal carrier state of man undergoing acclimation to heat. On the other hand, Miles, Williams, and Clayton-Cooper (*4*) state that the nasal carrier state varies not with the environment of the host, but with the host himself.

The correlation of changes in such factors as environmental temperature with alterations in the carrier state does not necessarily establish a cause and effect relationship. However, when one considers the delicate nature of the balance between host and microbe immediately prior to the appearance of overt disease, it is difficult to understand how the outcome of this interaction could be independent of the influence of external environment. This is especially true when such factors are known to produce physiological changes in the host associated with the stress phenomenon (*5*).

In this study two groups of Sprague-Dawley rats were exposed to different environmental conditions. The experimental group, consisting of 19 animals, was continuously exposed for 6 weeks to an ambient temperature of 5°C at a humidity of 90 percent. The control group of eight animals was maintained for a similar length of time at 25°C and 40-percent humidity. All animals were housed in individual metal cages and received Purina Laboratory Chow and water as desired.

Nasal cultures were obtained on each animal three mornings a week, on Monday, Wednesday, and Friday. The cultures were made by inserting a sterile flattened wire loop into the external nares, and then streaking the surface of prepared agar plates with the wire. The medium was coagulase agar (Difco), to which human plasma had been added in the ratio of one part plasma to nine parts agar (v/v). The plates were incubated for 24 hours at 37°C. Colonies of the *S. aureus* that fermented mannitol and produced coagulase were easily detected. The collecting loop was periodically streaked onto mannitol salt agar (Difco) to check results. In such instances, coagulase production was determined by the slide technique as described in the Difco manual (1958).

Table 1. The recovery of mannitol-fermenting and coagulase-producing colonies of *S. aureus* from rats exposed to cold for 6 weeks and those kept at room temperature.

Group	Total animals	Increased	Decreased	No change	Significance
Cold-exposed	19	15	1	3	<0.002
Control	8	1	4	3	>0.05

The number of cultures yielding mannitol-fermenting and coagulase-producing colonies to the total cultures taken was calculated for each individual rat. The number recovered for the first 3 weeks was compared with that of the last 3 weeks. There was an increase in the recovery of these potentially pathogenic colonies in 15 of the 19 animals exposed to cold and in only one of the eight control animals (Table 1). The increase for the cold group was highly significant by the Sign Test (*6*). There was only one animal from which mannitol-fermenting and coagulase-positive colonies were not recovered. This animal was one of the experimental group.

The results of this study would indicate that there is an effect of external environment upon the maintenance of the staphylococcal carrier state which should not be overlooked as a factor in the host-parasite relationship.

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