Technical Papers

Isomorphism of Terramycin and Aureomycin Hydrochlorides[1]

R. Pepinsky and T. Watanabe

Department of Physics,
The Pennsylvania State College, State College

Previous x-ray diffraction studies on terramycin hydrochloride in this laboratory (*1*) have established the cell size, space-group, and positions of a number of atoms in terramycin hydrochloride. An earlier report by Dunitz and Leonard (*2*) gave the cell and symmetry of aureomycin hydrochloride. The remarkable similarity of these cells suggested that the two compounds might be isomorphous, particularly since on the basis of published analytical data the two antibiotics might differ chemically only in the replacement of a hydroxyl group in terramycin by a chlorine in aureomycin.

If the two compounds did prove to be isomorphous, it is likely that phases of some Fourier coefficients for the electron density maps could be deduced by intensity comparisons. The presence of the additional chlorine in aureomycin would in any case be likely to provide more phase information than would be available from the terramycin measurements.

We have consequently taken single-crystal x-ray patterns of aureomycin hydrochloride and compared these with those previously obtained here for terramycin. The similarity in scattering is striking, and clearly establishes the isomorphism. The crystallographic constants are compared in Table 1.

TABLE 1

CRYSTALLOGRAPHIC COMPARISON OF TERRAMYCIN · HCl AND AUREOMYCIN · HCl

	Terramycin · HCl ($C_{22}H_{24}N_2O_9 \cdot HCl$) [*3*]	Aureomycin · HCl ($C_{22}H_{23}N_2O_8Cl \cdot HCl$)[2]
a	11.19	11.20 ± 0.02
b	12.49	12.89 ± 0.04
c	15.68	15.47 ± 0.02
Space group	$P2_12_12_1$	$P2_12_12_1$
d	1.51	1.5287
Mol wt (chemical)	496.7	515.1*
Mol wt (x-rays)	499 ± 5	514.5†

* Assuming Cl replacing OH of terramycin.
† The molecular weight calculated by Dunitz and Leonard (*2*) does not correspond with the formula they suggest and differs from our own determination.

The molecular weights determined from x-ray and density measurements are compatible with the assumed aureomycin formula.

Three-dimensional x-ray scattering data have been collected for aureomycin · HCl, using CuKα radiation and a Weissenberg camera, just as was done previously for terramycin · HCl. Comparison of Patterson projections for the two compounds, computed on X-RAC, has established the probable coordinates for the chlorine in aureomycin, which apparently replaces the hydroxyl in terramycin. Three-dimensional analyses are in progress. Location of the two chlorines per molecule, and probable location of some 11 lighter (nonhydrogen) atoms in terramycin, by the vector convergence density technique, now render the x-ray study very promising.

The isomorphism of these two compounds is not merely extremely helpful in the x-ray structure analysis; but poses intriguing problems in microorganism metabolism and antibiotic activity.[2]

[1] Development supported by Office of Naval Research, Contract No. N6onr26916, T. O. 16, and by a research grant from Chas. Pfizer & Co., Brooklyn, N. Y.

References

1. ROBERTSON, J. H., *et al. J. Am. Chem. Soc.*, **74**, 841 (1952).
2. DUNITZ, J., and LEONARD, J. *Ibid.*, **72**, 4276 (1950).
3. REGNA, P. P., and SOLOMONS, I. A. *Ann. N. Y. Acad. Sci.*, **53**, 229 (1950).

Manuscript received February 11, 1952.

[2] A private communication from J. D. Dunitz, received after this note was submitted for publication, reported a parallel observation of this isomorphism.

Physiology of an Infrared Receptor: The Facial Pit of Pit Vipers

Theodore H. Bullock and Raymond B. Cowles

Department of Zoology,
University of California, Los Angeles

Noble and Schmidt (*1*) in 1937 showed that the sense organ in the facial pit of blindfolded crotalids—rattlesnakes, copperheads, and moccasins—mediates the ability to strike correctly at moving objects such as a dead rat or a cloth-wrapped light bulb and to distinguish between warm and cold ones. They attempted to describe its sensitivity in terms of the reading of a mercury thermometer in the air at the position of the snake's head. However, it seems indicated by their conditions that radiant energy was the effective stimulus. We have undertaken to find out what can be learned of the function of this organ by recording activity from its nerves. The present paper is a preliminary report based on multi-unit analysis.

Specimens of several species of *Crotalus* have been curarized, and the superficial branch of the superior maxillary division of the trigeminal nerve has been exposed, tied, and cut proximally and lifted on electrodes. This is one of three nerves, all from the fifth cranial, ramifying in the thin membrane of the pit organ (*1, 2*). Equally good results have been obtained by a microneedle inserted into the membrane itself.

The nerve proves to be one of those afferents which carry a continuous barrage of impulses in the absence

of apparent stimulation—i.e., is "spontaneously" active. The activity is very complex in that not only nerve impulse spikes, but many slower deflections occur, and the average activity, integrated by a sound-level meter on "slow" setting (time constant = 0.7 sec), still shows fluctuations of 1 or 2 decibels.

Sound, odors, vibration of the substratum, touching the head and pit, and well heat-filtered light of moderate intensity (e.g., a match or flashlight at 5 cm) do not produce any detectable effect on nerve activity. But changes in the level of discharge are produced by mechanical deformation of the membrane, as with a wisp of cotton, by brushing the upper lip with a camel's hair brush (weak effect), by air movement, temperature changes of the body, and by common objects the temperature of which is above or below a neutral point, and which are brought into a receptive field around the face, regardless of intervening air temperature. Air movements must be abrupt, like weak puffs, and close to the face to be effective. Even so, many positions around the face yield no effect, and waving a thermoneutral object near the head is ineffective. Detection of air movement, then, is of doubtful importance in life as a function of this organ, although Noble and Schmidt interpreted some of their experiments on this ground.

Changing the temperature of the body or of the pit without altering the radiant energy impinging on the pit from the environment changes the maintained level of activity in the same direction, but without a high degree of sensitivity. In the extreme case, a change of 0.5° C and usually of 1 or 2 degrees is necessary to cause a detectable change in activity of the whole nerve. (Using a noise-level meter and integrating over several seconds, 1 decibel is about the limit of reliable change.) The organ could then function as an absolute temperature (as distinct from relative temperature) receptor if the brain chooses to use this information. The response—that is, the change in continual nerve activity—is greater at first to a sudden temperature change and then adapts in some seconds to the new level. This adapting fraction could serve as a more sensitive relative temperature receptor. Temperature effects of both types may really be responses to new levels of radiant energy falling on the membrane from the walls of the pit.

Outstanding in sensitivity compared to the above modalities is that to radiant heat. Any warm or any cold object causes a transient response with a threshold that can be roughly indicated by the human hand at 30 cm distance or by a glass of water 1° above or below a glass of water at the neutral temperature, held close to the pit. Since we are dealing with an entire nerve and a fluctuating background activity, it is possible that the threshold is actually much lower than appears from these observations. The neutral point is independent of body temperature and depends on the average radiation from all objects in the receptive field. Cold objects (relative to the field) depress nerve activity even if they are warmer than the body, and they are thus fully as noticeable to the snake as warm objects. Adaptation occurs and is complete except to very warm objects. Upon removal of the stimulus a large change in the opposite direction occurs. Thus an ice cube 5 cm from the pit causes a great inhibition, followed by adaptation, and upon its removal or screening, a large transient increase in nerve activity. The latency of these effects is usually 50–150 msec in the nerve 10 mm from the pit. Strong warm stimuli cause an "on" transient partially adapting to a new, higher level of discharge and an "off" transient in the opposite direction, or sometimes a long afterdischarge instead.

Moving a stimulating object within the receptive field causes noticeable alteration in nerve activity, and this is the only indication of distinction between stimuli, except quantitatively. If we keep the very rich innervation in mind, this suggests that there is localizing ability based on shadows cast on the deeply situated membrane by the margins of the pit. The receptive field (based on experiments with *Crotalus cerastes, C. ruber, C. viridis,* and *C. mitchelli*) is an irregular cone extending in the horizontal plane about 10° across the midline in front and almost at right angles to the body laterally from the pit. In the vertical plane lying obliquely between transverse and long axes and passing through the pit it extends forward only, and from about 35° below to about 45° above the horizontal.

Filter experiments and preliminary infrared monochromator experience indicate that the spectral sensitivity curve will show sensitivity to be extremely low to wavelengths shorter than 1 μ ("near infrared"), high in the region 2–3 μ and probably appreciable, if not high, into the long infrared as far as 10 or 15 μ. This longer end contains most of the energy radiating from objects like the human hand.

Block (*3*) suggested on the basis of its anatomy that this organ works like a Golay pneumatic radiant energy detector—i.e., by absorption of energy on a layer in the chamber behind the transparent membrane such as to result in a rise of temperature, expansion of gas, and consequent deformation of that membrane, the nerve endings detecting the deformation. Such instruments can be both sensitive and rapid. It is highly probable, however, that the pit membrane is opaque to the frequencies received. Moreover, we have experimentally cut the membrane, opening the posterior chamber to outside air, without losing responsiveness. Two other alternatives may be considered. The detection may be (*a*) by specialized molecules activated by the energy absorbed, as in the eye; or (*b*) by nonspecific absorption, resulting in a rise in temperature of the membrane, the nerve endings responding to this.

With respect to (*b*), on the quite improbable assumptions that all the energy received from a 0.1-sec exposure to a Nernst glower is absorbed and heats the 25-μ thick membrane uniformly, and that this has the heat capacity of water, we find that this stimulus at threshold distance in a certain snake would have produced a rise in temperature of the membrane of only

0.014° C; if only the first 7 μ were heated, which would probably include most of the nerve endings (*1, 2*), this figure would become 0.05° C. This is probably not small enough to rule out the possibility of mechanism (*b*), but the rather low sensitivity to temperature mentioned before makes this alternative seem unlikely.

With respect to hypothesis (*a*), there seems little possibility of a mechanism based on the usual photochemical reactions, such as those in the eye, since these are notable for their absence in this low-energy region of the spectrum (which is characterized by changes in vibrational and rotational energy levels as opposed to electronic changes, which are produced by absorption in the visible and ultraviolet). However, absorption bands in the infrared are assigned to specific atom groupings in organic molecules (*4*), so that specific activation of some kind may be possible.

References

1. NOBLE, G. K., and SCHMIDT, A. *Proc. Am. Phil. Soc.*, **77**, 263 (1937).
2. LYNN, W. G. *Am. J. Anat.*, **49**, 97 (1931).
3. BLOCK, M. J. *Nature*, **165**, 284 (1950).
4. RANDALL, H. M., *et al. Infrared Determination of Organic Structures*. New York: Van Nostrand (1949).

Manuscript received December 17, 1951.

A Virus Possibly Related to the Psittacosis-Lymphogranuloma-Pneumonitis Group Causing a Pneumonia in Sheep

D. G. McKercher[1]

School of Veterinary Medicine, University of California, Davis

A considerable number of sheep suffering from pneumonia are brought to this institution for autopsy. The absence of bacteria of etiological significance in many of the pneumonic lungs which were cultured suggested that in such cases a virus might be the cause, a possibility which has been recognized by others with respect to pneumonia of the ovine species. Accordingly, attempts were made to recover such an agent from these cases.

From 2 of 5 pneumonic sheep lungs an agent was recovered which induced a transmissible pneumonia in albino Swiss mice by intranasal inoculation. Impression smears prepared from the cut surfaces of the consolidated mouse lung, and stained according to Macchiavello's method (*1*), revealed the presence of elementary bodies which were morphologically and tinctorially identical to those of the psittacosis-lymphogranuloma-pneumonitis group (Chlamydozoaceae) of viruses. The agent was again recovered from the second sheep lung, which had been held under dry-ice refrigeration in the meantime, 3 weeks subsequent to the initial isolation. Repeated attempts by serial nasal inoculation in mice failed to recover any transmissible agent from the lungs of either the stock experimental mice or normal sheep.

[1] The author wishes to acknowledge his indebtedness to Joseph K. Saito for extremely able technical assistance; and to D. R. Cordy for helpful suggestions and for providing pathological materials for this study.

The mouse-lung-adapted virus was readily propagated in the yolk sac of 5- to 6-day-old embryonating hens' eggs, and was readily transmitted in serial passage. Subsequent to the first several yolk sac transfers, in which only the lower dilutions of inocula regularly proved infective, a 10^{-3} dilution of infected yolk sac regularly killed 100% of the embryos within 4–6 days following inoculation. In yolk sac smears, stained as described above, from moribund and dead embryos, large blue bodies were consistently present; these were assumed to be a developmental stage of the virus. Among them were occasionally observed the small red elementary bodies. A marked pneumonia was induced in mice by the inoculation of infected yolk sac suspension, and typical elementary bodies were regularly demonstrated in lung smears.

An inoculum was prepared from the supernatant obtained by centrifuging a 10% suspension of infected yolk sac at low speed for a short time. A vigorous 9-month-old wether was inoculated with 15 ml of this material, half being given intranasally and the remainder intratracheally. Mice also were inoculated. In addition, 3 kittens from which a transmissible respiratory agent could not be recovered by mouse lung passage of nasal washings, were given a portion of the supernatant by the nasal route. It was considered advisable to include kittens in order to determine whether the agent under study was identical with the feline pneumonitis virus of Baker (*2*) which, it was felt, might reside in the respiratory tract of sheep.

After inoculation the temperature of the sheep rose from a preinjection reading of 38.1° C to 41.2° C and dropped to the former level within 48 hr, where it remained for the next 6 days. Thereafter, until sacrifice of the animal on the 23rd day following inoculation, a fever was recorded which was characterized by intermittent peaks occurring at increasingly frequent intervals, and of progressively greater magnitude. During the 2-day period immediately preceding death the temperature constantly remained between 40.5° C and 41.0° C, and respiratory distress was noted. At autopsy, a pneumonia which, however, was not extensive, was found in the anterior lobes of both lungs, and the agent was readily recovered in mouse lung as described previously. Several of the inoculated mice died of the infection prior to the seventh day following inoculation, at which time the survivors were sacrificed. In all cases an extensive pneumonia had developed, and elementary bodies were readily demonstrated. The kittens, however, remained clinically normal during the 4-week period they were held following inoculation, and lung pathology was absent at autopsy. Attempts to recover the virus from the turbinate bones of these animals by serial passage in mouse lung were unsuccessful.

Urea-treated (*3*) infected and normal crude yolk sacs were used as antigens in complement fixation tests carried out with both homologous and feline pneu-