fuse illumination (A-type). These units were not completely unresponsive to light, but their reactions were extremely inconstant and weak; some of them, while not responding to long light stimuli, did respond to brief flashes of higher intensity. Only two units were found to be inhibited at "on" and "off" of the light (C-type). Some units responded to moving luminous spots in the visual field, as Hubel has seen in cortical neurons of the cat (*5*), but responses of this kind were not explored systematically.

Sixty-one units were unaffected by stimulating the brain stem, but the rest showed clear frequency changes in response to this stimulus, and the effects persisted for some time after its cessation. The firing of 14 units was accelerated, while that of 25 others was slowed down by reticular stimulation alone. Although there were exceptions, it was observed that light-activated units tended to be accelerated by the reticular stimulus, whereas units showing light inhibition tended to be likewise inhibited by the reticular stimulus (Fig. 2). In many such cases light and tegmental stimulation, when applied simultaneously, interacted with each other in mutual reinforcement. The majority of those units which did not respond to light were not affected by reticular stimulation. Discharges induced by darkness, prominent in most D- and E-units at the cessation of light, were in some instances enhanced and in others inhibited by brain-stem stimulation. No correlation was found between cortical depth and types of response to either light or electrical stimulation.

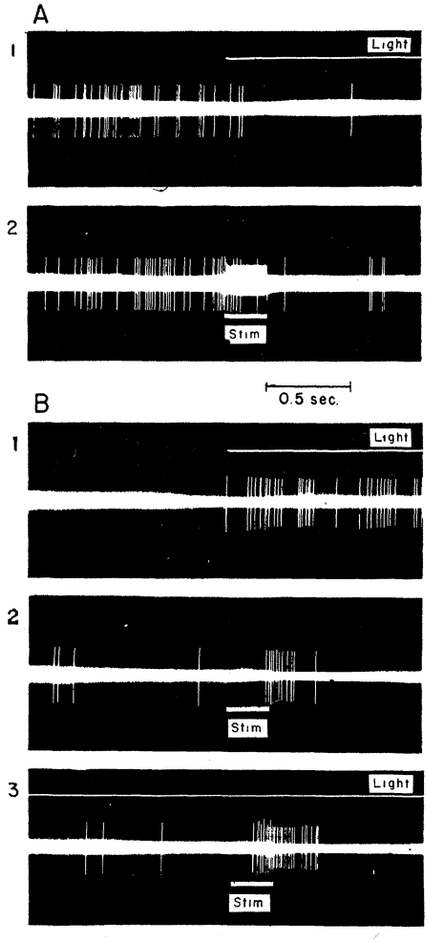

Fig. 2. Record excerpts from two single units: (*A*) *1*, light-inhibited unit; *2*, same unit in dark, inhibited by reticular stimulus. (*B*) *1*, light-activated unit; *2*, same unit activated by reticular stimulus alone; *3*, same unit, reticular stimulus during light. Records read from left to right.

This study demonstrates that reticular activation can result in excitatory or inhibitory modulation of certain neurons in the striate cortex. Furthermore, it shows that this modulation acts in many instances synergically with luminous stimuli, although more work is needed to determine how this synergism is accomplished. Since both excitatory and inhibitory synaptic effects have been demonstrated in cortical cells by stimulation of the lateral geniculate body (*8*), it is possible that the reticular influences reported here are exerted upon presynaptic neurons at the geniculate or intracortical levels.

It has been postulated (*1*) that the same basic process that secures arousal from sleep—that is, the generalized activation of the cortex by the reticular core of the brain stem—is responsible in the awake organism for the attainment and maintenance of states of high receptivity of the sensory cortex. Thus, such a mechanism might underlie a descent of sensory thresholds in behavioral alertness. Some support for this postulate is provided by the fact that reticular activation, experimentally elicited, has differential effects upon various visual cortical cells according to the characteristics of their responses to optic stimuli. It is recognized that these characteristics are determined by the nature of the receptive fields of the individual units, the spatial configuration of stimulus intensities, and other possible factors which account for the complexity of the cortical representation of visual patterns.

The probably ubiquitous character of reticular activation upon sensory areas does not preclude the existence of more specialized processes, perhaps corticothalamic, conceivably operating to channel reticular tonus, and that may be at the basis of selective focusing of attention.

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References and Notes

1. J. M. Fuster, *Science* **127**, 150 (1958). A complete study is in preparation.
2. This work was supported by grants (M-2411 and M-3756) from the U.S. Public Health Service.
3. O. Creutzfeldt and H. Akimoto, *Arch. Psychiat. Nervenkrankh.* **196**, 520 (1958).
4. J. D. Green, *Nature* **182**, 962 (1958).
5. D. H. Hubel, *J. Physiol. London* **147**, 226 (1959).
6. J. M. Fuster, unpublished observations.
7. R. Jung, O. Creutzfeldt, O.-J. Grüsser, *Deut. med. Wochschr.* **82**, 1050 (1957).
8. C.-L. Li, A. Ortiz-Galvin, S. N. Chou, S. Y. Howard, *J. Neurophysiol.* **23**, 592 (1960).

13 February 1961

Auxetic Growth in the Javanese Toad, Bufo melanostictus

Abstract. Morphologically, it has been found that erythrocyte size in the Javanese toad is greater in large than in small animals, and preliminary data indicate that the same is true of kidney, intestinal, and liver cells. Physiologically, the hemoglobin concentration, packed cell volume, specific gravity of the whole blood, and the liver glycogen concentration also increase with the size of the animals.

One of the advantages of working with tropical amphibians on Java springs from the fact that the unchanging climatic factors, especially temperature and humidity, and the unending food supply, make it possible for them to maintain constant reproductive and physiological conditions throughout the year. These conditions remain unchanged in spite of the fact that the majority of amphibians in the Indonesian Archipelago have migrated there from northern areas where they are known to undergo seasonal physiological and reproductive cycles as do other temperate-zone amphibians.

It has recently been shown by Church (*1*) and his co-workers (*2*) that in *Bufo melanostictus* and *Rana cancrivora* the reproductive patterns and underlying physiological mechanisms governing them, as indicated in the storage of liver glycogen, fat bodies, hemoglobin concentrations, and pituitary sizes and secretions, have been altered from the temperate-zone norms to favor a more

complete adaptability of the animals to the unchanging tropical environment into which they have migrated.

From these studies it was found that not only the reproductive physiology, but also the growth of the animals, was affected directly or indirectly by the unchanging climate.

It is a well-known fact that a poikilothermous animal will continue to grow throughout its lifetime, and size thereby becomes a measure of age. It is usually assumed that this growth and accretion of bulk is attained for the most part through the mitotic increase of cells, although Donaldson (*3*) has shown that in the cerebral cortex of the rat the number of cells increases until about 20 days after birth, after which the subsequent increase in the weight of the brain is due largely to increase in the size of the cells; and Ott (*4*) reported that rat muscle increases in size in much the same way. A comparable auxesis has been found to occur in *Bufo melanostictus*.

In a study of the blood of *B. melanostictus*, erythrocytes have been found to increase in size continuously throughout the life of the animal. The sizes of the cells were determined by planimetric measurements of camera lucida drawings of blood smears taken from animals varying in size from tadpoles showing hind-limb buds just beginning to develop, through metamorphosis, to the largest adults available (snout-vent length approximately 90 mm). In all, the blood of 16 groups, representing 160 animals, was examined. Ten cells of each of ten animals in each group, totaling 100 cells per group, were drawn and measured.

When the mean size of the erythrocytes was plotted against the mean snout-vent lengths of the animals in a group from which the blood was taken, the size of the erythrocytes was found to increase with the size of the animal, those of the largest adults attaining a size twice that of a small tadpole (Fig. 1). Wintrobe (*5*) has reported varying erythrocyte size in different amphibian species, and Fankhauser (*6*) has shown variations in cell size to occur in heteroploid salamanders. But, except for a decided decrease in size when the animals left the water after metamorphosis, these results show a continuous increase of erythrocyte size with age in normal specimens within the same species (Fig. 2).

Work in progress indicates that perhaps all somatic cells in *B. melanostictus* continue to grow auxetically throughout life, and one of the interesting aspects of the investigation is that not only the morphology of the animals is involved but also their physiology.

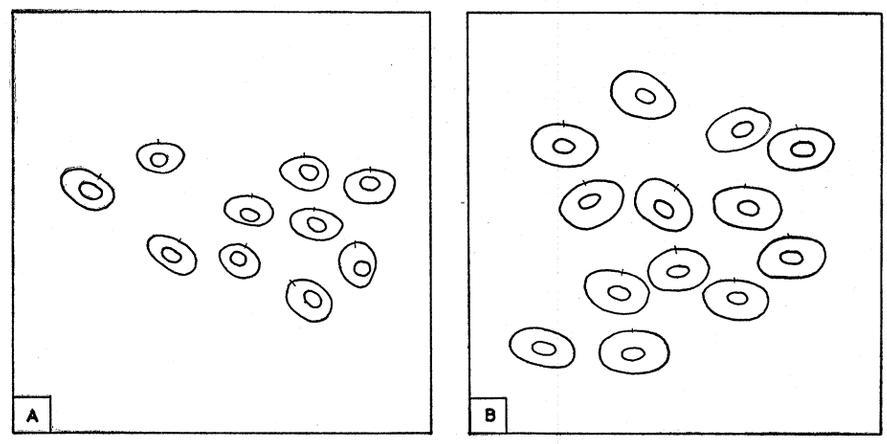

Fig. 1. Camera lucida drawings showing size differences between (*A*) the erythrocytes of a *Bufo melanostictus* tadpole with limb buds just beginning to appear and (*B*) the erythrocytes of a large adult with a snout-vent length of 90 mm (about × 210).

Preliminary studies indicate that kidney cuboidal epithelial cells and intestinal columnar epithelial cells continue to increase in size as the animals grow larger. The same may also prove true for liver cells.

Physiologically, in spite of the fact that the erythrocyte count in *B. melanostictus* remains more or less constant regardless of size, the hemoglobin concentration, packed cell volume, and the specific gravity of the whole blood also increase with the size of the animals

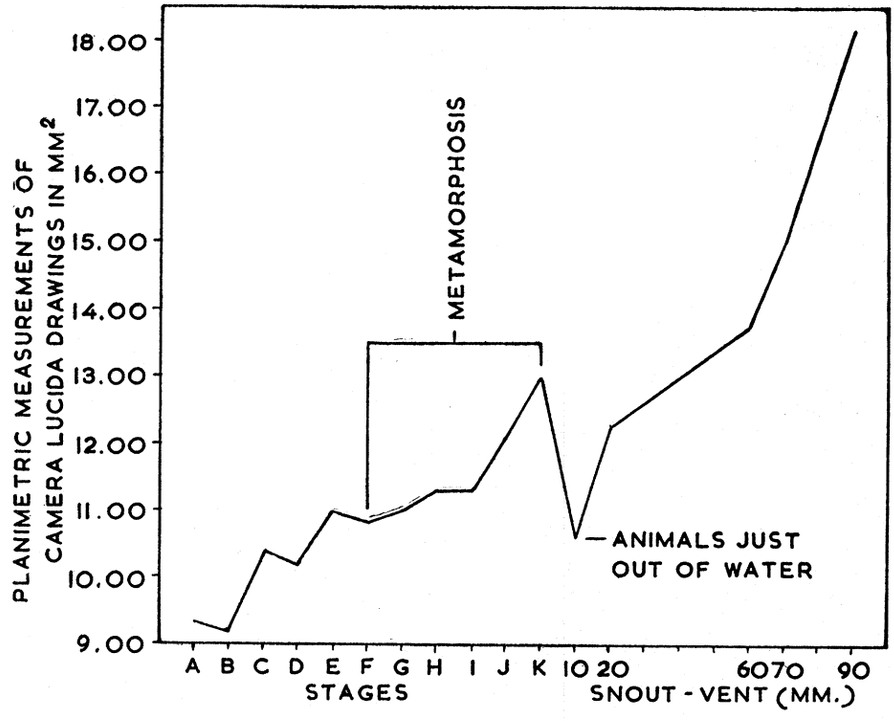

Fig. 2. Auxetic growth of the erythrocytes of *Bufo melanostictus*. *A* to *K* represent stages in tadpole development; 10 to 90 mm are the snout-vent lengths of metamorphosed animals. Tail resorption began at stage *F* and metamorphosis was completed at stage *K*. The animals had a snout-vent length of 10 mm when they left the water. Except for a decrease in the size of erythrocytes when the animals left the water, which was perhaps due to dehydration, the erythrocytes continued to increase in size until in the largest adults they were almost twice as large as in the smallest tadpoles.

(*7*). In females, the picture is complicated by a further increase in hemoglobin concentration and specific gravity accompanying the maturity of the ovarian eggs. In the same way, the glycogen concentration of the liver is higher per gram of tissue in larger animals than in small (*8*).

All in all these results show that as auxetic growth continues, concomitant physiological changes occur which probably affect the metabolism of the animal. To clarify this conclusion, the effects of environmental changes in temperature, dehydration, and diet, and possible changes in the serum proteins, are being investigated in addition to the studies mentioned above.

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References

1. G. Church, *Zoologica* **45**, Pt. 4, 13 (1960); *Treubia* **25**, 2 (1960).
2. G. Church, D. T. The, K. T. Sie, J. Kusin, K. L. Tio, M. Hussaini, *Indonesian Council for Sciences*, Djarkarta (1958).
3. H. H. Donaldson, *The Rat* (Mem. Wistar Inst. Anat. and Biol. No. 6, Philadelphia, 1925).
4. O. Ott, *Biol. Generalis* **12** (1937).
5. M. M. Wintrobe, *Folia Haematol.* **51** (1933).
6. G. Fankhauser, *J. Exptl. Zool.* **100** (1945).
7. G. Church and M. Hussaini, unpublished data.
8. G. Church and K. T. Sie, unpublished data.

27 January 1961

Synthesis of Bacterial Cellulose from Labeled Precursor

Abstract. The isolation and purification of an immediate precursor of bacterial cellulose was confirmed with glucose randomly labeled with carbon-14. The glucose appears to be bound within the cell to a lipid, is carried across the bacterial cell wall, and is incorporated enzymatically into cellulose extracellularly.

The isolation and purification of a compound containing glucose bound to a lipid, from ethanol extracts of active suspensions of *Acetobacter xylinum*, has been described recently (*1*). This compound appears to be the precursor of bacterial cellulose in that it will form typical microfibrils in aqueous solutions containing an extracellular enzyme. Preparations of the compound were shown to be homogeneous by two-dimensional chromatography in an acidic and a basic solvent, as tested by four detector systems, including autoradiography. We report here confirmation of the previous work from the results of a study of the transfer of glucose randomly labeled with C^{14} from the above compound to cellulose, under conditions which simulate in vivo formation of microfibrils. These results afford additional insight into the mechanism of biosynthesis of cellulose by this bacterium.

Labeled precursor was prepared as follows: Cellulose-free, washed cells from 100 ml of a suspension of *A. xylinum*, prepared as described previously (*2*), were incubated for 4 or 5 min (depending on the activity of the individual cell suspension) at 35°C in a 2 percent glucose solution, 0.01*M* in phosphate buffer, *p*H 6.0. Sufficient glucose in this solution was randomly labeled with C^{14} to give radioactivity of 2.5 μc/ml of initial solution. The isolation of the precursor from ethanol extracts of these suspensions, in the fourth fraction off a magnesium trisilicate-Celite column, M4, was carried out as reported earlier (*1*). For autoradiography of the labeled preparations, paper chromatograms (acid-washed Whatman No. 1 paper 7 by 7 in.) were developed two-dimensionally by the descending technique in *n*-butanol, acetic acid, and water (4:1:1) and *n*-butanol, pyridine, and water (10:3:3). The chromatograms were then placed in contact with medical x-ray film, which was developed after 4 days of exposure. Quantitative estimates of the fraction of glucose incorporated into the precursor at any instant were not undertaken because the fraction is very small and the compound is sensitive to traces of water on the paper. For instance, drying the preparation on initially slightly damp chromatographic paper will completely destroy it.

The fraction containing the precursor of bacterial cellulose, M4, exhibited only one spot on the autoradiograms in the same position as the compound detected previously (*1*) by KIO_4-starch reagent (*3*). At low concentrations round compact spots were observed, but at higher concentrations severe streaking of the chromatogram was always present. This streaking is attributed both to a slow breakdown of the compound in the water phase and to a slow rate of attainment of equilibrium. No traces of the predominant compounds in the suspension medium, glucose, and the gluconates were detectable in the autoradiograms of the M4 fraction.

The incorporation of a component of M4 fraction into bacterial cellulose, under certain conditions, was demonstrated as follows: (i) 1 ml of M4 incubated with 1 ml of water for 30 min at 35°C; (ii) 1 ml of M4 incubated as above with 1 ml of an ultrafiltered supernatant fraction of an active culture which contains an enzyme catalyzing cellulose formation (*4*); (iii) 1 ml of ultrafiltered supernatant fraction incubated alone for 30 min at 35°C. After incubation, each sample was diluted to 10 ml with redistilled absolute ethanol and centrifuged at 15,000 *g* for 15 min. The pellet, if any, was re-extracted with 3 ml of chloroform to remove lipids and recentrifuged.

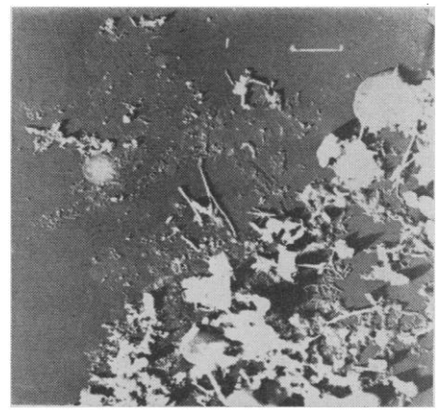

Fig. 1. Typical chloroform-ethanol insoluble, alkali-resistant, amorphous material formed by M4 fraction in the presence of only water.

Ethanol-chloroform insoluble material was formed principally in (ii), very little in (i), and none in (iii). The pellets from (i) and (ii) were digested with hot 4 percent NaOH to remove noncellulosic polymers (*4*), washed free of NaOH, dispersed in water,

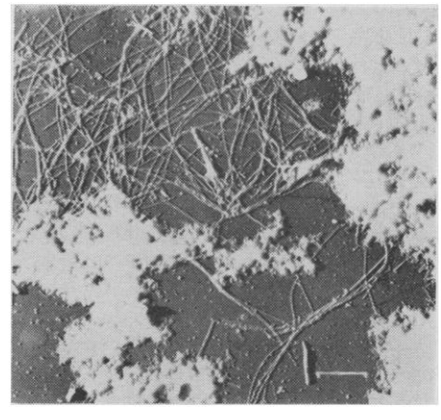

Fig. 2. Typical chloroform-ethanol insoluble, alkali-resistant cellulose microfibrils formed by the M4 fraction in the presence of an enzyme in the ultrafiltered supernatant fraction of an active culture of *A. xylinum*. Note the presence of substantial amorphous material also.