liefs are not limited to any one religious creed, ethnic group, age level, or degree of educational achievement. Among the believers in Pele are some highly educated individuals and some prominent citizens of the island.

The old Hawaiian beliefs are remarkably rational and provide an apparently consistent explanation for geological growth processes. The beliefs appear to be reinforced by a number of factors. One such factor is the very sight of the lava fountains which are often over 1000 ft in height (see Fig. 2). This magnificent phenomenon is accompanied by auditory, olfactory, and tactile stimulation produced by the fountain and its accompanying fallout.

Another source of reinforcement for belief in Pele is the inability of science and technology to cope with the destructiveness of the lava flows. The building of dikes to contain or divert the lava appears to be uniformly unsuccessful.

The nature of the beliefs in Pele and the source of their perpetuation are being systematically investigated. Belief in Pele is openly acknowledged by many of the evacuees.

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Note

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A Nonsteroidal Androgen

Abstract. 2-Acetyl-7-oxo-1, 2, 3, 4, 4a, 4b, 5, 6, 7, 9, 10, 10a-dodecahydrophenanthrene possesses androgenic activity when applied directly to the chick's comb. This action is in addition to the antiandrogenic activity previously reported for this compound. This is the first demonstration of androgenic activity by a compound not possessing the steroid nucleus.

Up to the present time androgenicity has always been associated with compounds possessing the steroid nucleus or those having only minor modifications in the nucleus, such as the *D*-homo analogs of testosterone (*1*). It has now been demonstrated that a perhydro phenanthrene derivative, previously reported to be an antiandrogen in rats (*2*) and in chicks (*3*), also exhibits androgenic activity when applied directly to the chick's comb.

Table 1. Androgenic activity of Ro 2-7239 inthe chick, when inuncted in absolute alcohol to the comb.

Material inuncted	Total dose (μg)	No. of chicks	Mean comb ratio ± S.E.
	Experiment A		
None	0	18	0.39 ± 0.021
Testosterone	1	14	0.36 ± 0.016
Testosterone	3	14	0.48 ± 0.028
Testosterone	9	13	0.59 ± 0.038
Testosterone	27	13	0.97 ± 0.052
Ro 2-7239	1	14	0.42 ± 0.024
Ro 2-7239	10	14	0.42 ± 0.024
Ro 2-7239	50	13	0.50 ± 0.029
Ro 2-7239	250	14	0.52 ± 0.033
Ro 2-7239	1000	12	0.56 ± 0.034
	Experiment B		
None	0	15	0.33 ± 0.018
Testosterone	1	14	0.43 ± 0.019
Testosterone	4	15	0.47 ± 0.027
Testosterone	16	12	0.68 ± 0.047
Ro 2-7239	10	12	0.42 ± 0.018
Ro 2-7239	100	12	0.47 ± 0.024
Ro 2-7239	500	13	0.60 ± 0.036

Table 1 documents typical results on the influence of Ro 2-7239 (2-acetyl-7-oxo-1, 2, 3, 4, 4a, 4b, 5, 6, 7, 9, 10, 10a-dodecahydrophenanthrene) on the chick's comb when applied directly to it. For this test, 1-day-old white leghorn male chicks were inuncted once daily with 0.05 ml of an absolute alcohol solution of the test compound, the standard testosterone, or solvent alone. After seven daily inunctions, the combs were removed and weighed to the nearest 0.5 mg, and the results were expressed as the ratio of comb weight in milligrams to body weight in grams.

Statistically significant increases ($P = 0.01$) in the comb ratios were found at dose levels of 50, 250, and 1000 μg in experiment A and at 10, 100, and 500 μg quantities in experiment B for Ro 2-7239. The comb response to Ro 2-7239 was strikingly different from that found for testosterone. Although relatively small doses produced significant growth of the comb, increasing the amount inuncted on the comb by a factor of 20 produced only minor further increments (*4*).

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4. I am grateful to Theresa Rutkiewicz and Albert Tomolonis for valuable technical assistance. This work was supported in part by U.S. Public Health Service grant No. CY-2193 and by a grant from Hoffmann-LaRoche, Inc.

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Nervous Regulation of Conditioned Hyperglycemia to Nociceptive Stimulation

Abstract. A conditioned rise in blood sugar occurs in rats during a 10-minute waiting period prior to nociceptive stimulation. This conditioned "preparatory hyperglycemia" is abolished after a bilateral lesion is produced in the mid-line thalamic nuclei; the experiments point to the importance of the thalamic reticular formation in adaptative metabolic reactions.

Cortical and subcortical mechanisms apparently participate in the nervous regulation of blood-sugar levels. The importance of mechanisms of higher nervous activity has been shown by the method of conditioned reflexes (*1*); evidence concerning the importance of subcortical regulation is, however, less clear. Interest had been concentrated especially on the influence of hypothalamic nuclei in experiments in which stimulation (*2*) or lesions (*3*) of these nuclei were used.

With increasing evidence concerning the role played by nonspecific brainstem mechanisms in the establishment of conditioned reflexes (*4*), information has also been obtained indicating the importance of the activation of the reticular system on homeostatic processes (*5*), the action of adrenalin as a powerful stimulant of ascending and descending reticular facilitating systems being of special interest in this connection (*6*). Experiments in which the conditioned hyperglycemia that precedes nociceptive stimulation is studied offer a new approach for investigation of both aspects of the function of the reticular system in adaptative behavior, through study of the increasing alertness and the increase in blood-sugar levels which occur when animals are repeatedly conditioned to a nociceptive stimulus.

Rats were put into a cage, and after a "waiting period" of 10 minutes, electrical shocks were applied through the floor of the cage. Blood-sugar levels were then determined, before and at the end of the "waiting period," before the nociceptive stimulus was applied. No increase in blood sugar was found after the first exposure, but there was a statistically significant rise in blood glucose during the period of conditioning to nociceptive stimulation—that is, a "preparatory hyperglycemia" usually appeared after the third conditioning period. This conditioned metabolic reaction to nociceptive stimulation was accompanied by a considerable decrease in motor activity, this being especially marked at the end of the "waiting period."

In further experiments the development of this preparatory hyperglycemia reaction was studied in animals in which bilateral lesion of the mid-line thalamic

nuclei (the so-called nuclei of the paleothalamus) was performed. These nuclei of the nonspecific thalamic system appear to be the diencephalic prolongation of the brain stem-reticular system, and it is probable, since they play a role in reactions of alertness, that they also participate in "preparatory reactions" to nociceptive stimulation.

In animals in which a clear "preparatory hyperglycemia" was elaborated after 14 conditioning periods, a bilateral lesion of the thalamic mid-line nuclei was produced by the stereotactic method. Three days later the animals were again put into the cage, and blood-sugar levels were determined before and at the end of the 10-minute waiting period. No rise in blood sugar was observed in these animals (Fig. 1).

The center of the lesion was found to be localized in the thalamic mid-line nuclei in all 14 cases in which the lesion was produced (Fig. 2), but there was also some slight peripheral disturbance in adjacent nuclei, especially of the hypothalamus. In the 14 animals in which the lesion was performed the mid-line nuclei of the thalamus were destroyed in all cases. There was no disturbance of the ventral dorsomedial nucleus of the thalamus. Slight peripheral disturbance was found in the hypothalamic dorsal nucleus in 13 cases, of the paraventricular nucleus in three cases, of the anterior hypothalamic nucleus area in four cases, and of the ventromedial part of the thalamus in three cases.

This loss of the conditioned "preparatory reaction" to nociceptive stimulation is not due to the operative procedure. In control animals with elaborated "preparatory hyperglycemia" prior to nociceptive stimulation, a needle was introduced into the region of the mid-line thalamic nuclei by the stereotactic method, but no electrolytic lesion was produced. In these cases it was found, in tests 3 days later, that the hyperglycemia had not been lost. In another series of control experiments in animals with elaborated preparatory reaction to nociceptive stimulation, a bilateral stereotactic lesion was performed in other brain regions—that is, in anterior parts of the brain. Again, the hyperglycemic preparatory reaction was not affected; this indicated a certain specificity of the thalamic mid-line nuclei in the elaboration of the "metabolic preparatory response."

The assumption of a nonspecific disturbance of higher nervous system activity cannot explain this finding since animals in which a conditioned "avoidance" reaction to nociceptive stimulation has been elaborated retain this reaction after a lesion of this area with electrocoagulation.

We may therefore conclude that the thalamic mid-line nuclei, in which the reticular ascending system has an important relay station, are of great importance in the elaboration of the conditioned hyperglycemic preparatory reaction to nociceptive stimulation.

Afferent signals apparently activate these thalamic nuclei, and it is probable

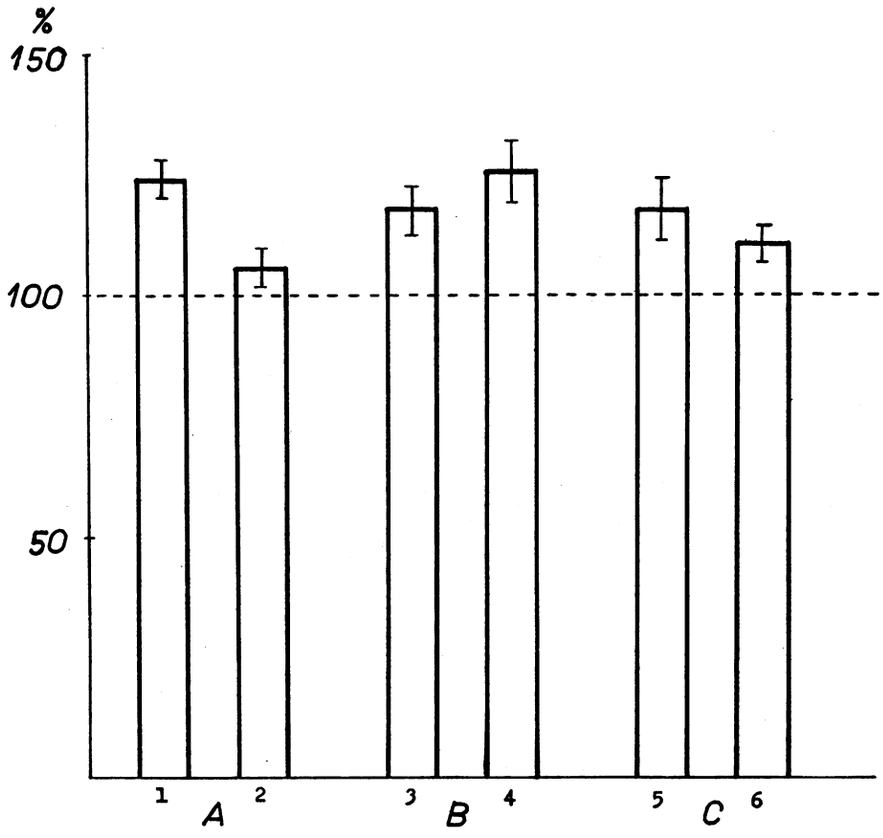

Fig. 1. Rise in blood glucose (in percent) in rats during a 10-minute waiting period prior to nociceptive stimulation; 100 percent is the initial level of blood glucose before the start of the waiting period. In rats that waited repeatedly for a period of 10 minutes prior to nociceptive stimulation, a "preparatory hyperglycemia" developed. The graph shows the rise in blood glucose (in percent) during the waiting period before (1) and after (2) a bilateral lesion of the thalamic mid-line nuclei (*A*); before (3) and after (4) introduction of a stereotactic needle without production of an electrolytic lesion (*B*); and before (5) and after (6) a lesion in the rostral part of the brain (*C*).

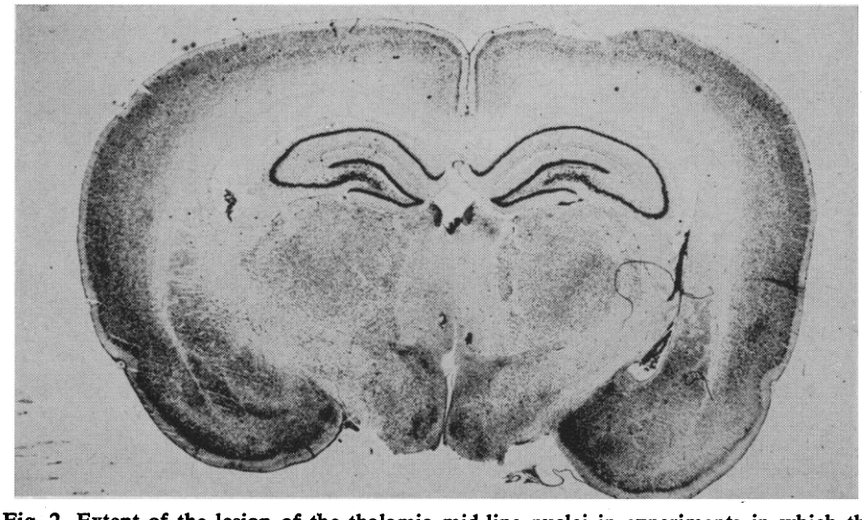

Fig. 2. Extent of the lesion of the thalamic mid-line nuclei in experiments in which the "preparatory hyperglycemia" reaction to nociceptive stimulation was abolished after the lesion had been produced.

that afferent pathways from the diencephalic reticular ascending system induce, via hypothalamic centers, neurohumoral mechanisms participating in stress situations, including increased liberation of adrenalin. The lesion of the thalamic mid-line nuclei apparently interrupts this neurohumoral reflex arc which participates in metabolic preparatory reactions.

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23 November 1959

Synchronization of Division in Escherichia coli

Abstract. The smallest cells of a culture in the logarithmic phase, when isolated by a single rapid filtration through 1.2-μ Millipore paper, show good synchronization and reproducibility through the first division cycle. The method minimizes metabolic shock and provides a culture in which an easily reproducible initial state is established at a known time.

Tests of the Maruyama and Yanagita technique (*1*) for isolating the largest cells of an *Escherichia coli* culture led us to the conclusion that temperature shock, introduced by extensive manipulations at room temperature, was probably responsible for the erratic results obtained. Subsequent experiments led to a synchronizing technique which is simple and fast and involves no operations likely to disturb the normal metabolism of the organism.

Our procedure for *E. coli* strains K12 (λ) and B is as follows (*2*). A 100- to 150-ml batch culture is grown in synthetic medium (*3*), with continuous aeration, to a density of the order of 10^8 cells per milliliter. Without other manipulation, the culture is filtered quickly through a single sheet of grade RA Millipore paper, pore size 1.2 μ, standard Millipore equipment being used for vacuum filtration (*4*), with a 25-liter bottle as "vacuum reservoir." Filtration is interrupted when a few milliliters of culture remain on the filter or when clogging reduces the rate of filtration appreciably. Filtration is completed in 2 to 3 minutes. The filtrate, containing 1 to 2 percent of the total cell population, is aerated through a sintered glass wand immediately after filtration. The entire process is carried out in the constant-temperature cabinet in which the initial culture is grown. No significant improvement resulted from repeated filtration through single sheets of Millipore paper or from the use of stacks of two or more papers.

Figure 1 shows the results of a typical experiment in which the filtrate was sampled periodically and assayed for cell count by triplicate plating on nutrient agar. Dilutions were adjusted to yield about 100 colonies per plate at minimal density. Zero time is taken at the mid-point of the filtration interval. The results of all our tests, with the two strains of *E. coli* and temperatures ranging from 25° to 37°C, may be summarized as follows. (i) Through the first growth cycle the $n(t)$ curve (cell count–time curve) is flat for about 70 percent of the mean generation time. The doubling time approximates the mean generation time closely. Contrary to common experience with temperature shock methods, the doubling is real rather than nominal, supporting the conclusion that quick filtration without separation of the cells from the original medium involves little if any disturbance of normal growth. (ii) Under close temperature control the second division cycle is well marked, but synchronization deteriorates in this cycle. Beyond 1.5 times the mean generation time, reproducibility of the $n(t)$ curve has not been good enough to warrant selection of a typical curve. Relaxation of temperature control is reflected in a deterioration of synchrony and reproducibility in the interval from 0.7 to 1.5 times the mean generation time. (iii) Beyond the second cycle the $n(t)$ curve assumes a form which approximates the normal growth curve.

As additional grades of Millipore paper become available, improvement in cell-size resolution, with consequent sharpening of synchronization, will probably be possible. It should be remarked, however, that if the standard deviation in generation time characteristic of *E. coli* is as large as 0.3, as was recently estimated (*5*), a cell population strictly homogeneous in age at filtration would show rapid deterioration of synchrony after the first cycle. Attempts made in our laboratory by W. V. Morgan and one of us

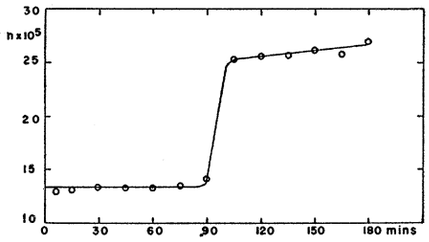

Fig. 1. Cell count-time characteristic for *E. coli* K12 (λ) filtered at zero time. *N*, cells per milliliter; *t*, 30°C; mean generation time, 105 minutes. Grade RA Millipore paper was used as filter.

(P.A.A.) to impose synchronized division on continuously cultured *E. coli* by programmed temperature cycling gave disappointing results which are probably attributable to this factor.

From the preparative standpoint, the quick-filtration method would appear to have the marked advantage of supplying a culture for which the initial state and succeeding growth curve are sharply defined on the time axis and hence determinable before rather than after the event (*6*).

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Utilization of Organic Carbon by a Marine Crustacean: Analysis with Carbon-14

Abstract. An isotope-dilution technique using carbon-14 was employed to determine quantitatively the carbon budget of a filter-feeding crustacean. The amount of carbon ingested ranged between 0.044 and 0.139 mg. Incorporation of carbon varied between 11.3 and 73.6 percent per day per organism, with an average of 32.5 percent for the animals tested. Values for oxygen consumption are given as they relate to carbon intake and utilization.

Energy and feeding interrelationships have been experimentally determined for some zooplankters. For example, Richman (*1*) determined a calorimetric budget for the fresh water crustacean