Reports

Graying of Hair in Rats Fed a Ration Deficient in Lysine

As early as 1914, Osborne and Mendel demonstrated the essential nature of lysine for the growth of rats that were fed a diet containing zein as the sole source of protein. The rats were maintained at an almost constant body weight for 182 days, and they also showed no growth of hair (*1*). This result has been confirmed further by the studies of Rose (*2*) in which purified rations with mixtures of amino acids to simulate the composition of protein were supplied to rats. Smuts *et al.* (*3*) have also verified that lysine deficiency inhibits the body as well as the hair growth, but found no difference in the cystine content of the hair of normal and lysine-deficient rats.

In turkey poults, a deficiency of lysine has been shown to cause depigmentation of feathers (*4*); this implies an interference in the synthesis of melanin. It is of interest to find whether lysine deficiency has any effect on the graying of hair in a mammal such as the rat.

In the earlier studies, albino rats were used for determining lysine requirements and any graying tendencies would have been very difficult to notice. For this reason, black rats of the Long-Evans strain which are maintained in the animal husbandry department at the University of California, Davis, were used.

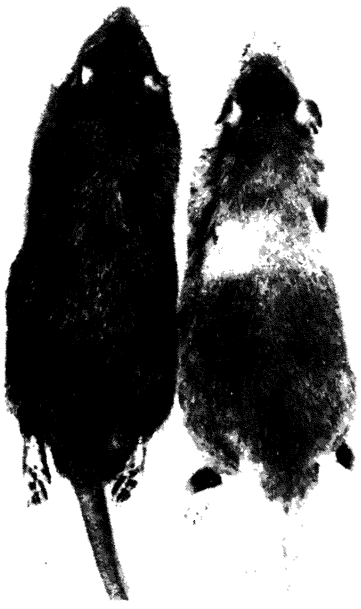

Fig. 1. Rats fed lysine-deficient and supplemented diets. Note the lighter coat color of the deficient rat on the right.

Five black male rats, weaned at about 21 days of age (not necessarily litter mates) were divided into two groups, A and B of 3 and 2 animals, respectively, and were maintained on a gluten-basal diet for a period of 25 days. Each 100 g of this ration contained the following components: glucose, 62.6 g; gluten (81 percent protein), 25.0 g; soybean oil, 5.0 g; HMV salt mix, 4.0 g; vitamin mix (Vohra and Kratzer, *5*), 2.0 g; choline chloride (25 percent), 0.8 g; DL-tryptophan, 0.12 g; DL-methionine, 0.1 g; dry vitamin A (10,000 units/g), 0.1 g; dry vitamin D_3 (1,500 units/g), 0.1 g; dry vitamin E (44 units/g), 0.1 g; biotin, 0.02 mg; and vitamin B_{12}, 0.001 mg. The rats were weighed two times a week and were found to gain an average of 0.8 percent of their body weight per day. After 25 days, the control group A was fed a sesame-basal diet (Vohra and Kratzer, *5*) which was slightly deficient in lysine and which has caused a depigmentation in turkey poult feathers. Group B was given the sesame-basal diet supplemented with 1.1 percent DL-lysine, which has proved to be adequate in preventing the depigmentation of poult feathers. Again, the feed and water were supplied *ad libitum*. A portion of the back of each rat was shaved to facilitate noting the change in color of the growing hair.

The sesame-basal diet produced growth of 3.5 percent daily gain, which was much more rapid than that produced by the gluten-basal ration, although it was not optimal. The addition of 1.1 percent of DL-lysine improved the growth of the rats to 4.1 percent daily gain.

The most noticeable difference in the two groups was in the color and texture of the hair coats (Fig. 1). The coats of the lysine-deficient rats were very much lighter in color and finer in texture than the dark black and coarse hair coats of the lysine-supplemented rats. This experiment has been repeated three times, and lysine deficiency has caused a graying of the hair each time.

From these experiments it may be concluded that lysine plays some role in the synthesis of melanin in the hair of rats as well as in the feathers of turkey poults.

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Spontaneous Rewarming of the Hypothermic Curarized Dog

That striated muscle plays a leading role in the regulation of body temperature is a widely accepted concept. It is not certain, however, that this is the only source of heat production which is actively regulated in the process of maintaining normal body temperatures or whether there are other heat-producing mechanisms that are subject to thermostatic control.

Many years ago it was postulated that either shivering or chemical regulation or both were involved in maintaining normal temperature levels in a cold environment (*1*). The term *chemical regulation* was never made clear but was intended to represent a rise in metabolism due neither to muscular activity nor to increased muscular tension (*2*). Chemical agents or calorigenic hormones or both were considered possibly responsible. The findings of increased oxygen consumption and succinoxidase activity in liver tissue after chronic exposure of rats to cold have been presented as a direct demonstration of increased metabolism of visceral tissue. The mechanisms of these increases were not made clear (*3*).

DuBois (*4*) believed it quite possible that imperceptible tensing of muscles could account for all so-called "chemical regulation"; Burton and Bronk (*5*) and Sellers *et al.* (*6*) demonstrated that there was increased muscular tension and electric activity with resultant increased oxygen consumpion before shivering became overt. Following acute exposure to cold of dogs anesthetized with thiopentone, Ross and Werner (*7*) believed that they observed an initial spontaneous re-

Table 1. Rates of cooling and rewarming, rectal temperatures, and oxygen consumption of curarized dogs cooled in an ice and water bath. Averages are given below the short rules.

Dog weight (kg)	Control temp. (°C)	Temp. when removed from bath (°C)	Rate of cooling (°C/min)	Lowest temp. recorded (°C)	O_2 consumption at control temp. (cc/min)	O_2 consumption at lowest temp. % of control (%)	Rate of rewarming to 30°C (°C/hr)	O_2 consumption at 30°C % of control (%)
20.2	36.7	29.0	0.32	26.0	160	31	0.69	47
18.2	36.0	29.0	0.25	23.7	120	21	0.63	50
18.0	33.3	28.3	0.24	25.1	110	35	0.31	68
14.8	36.7	28.7	0.36	25.7	132	39	0.86	44
15.8	34.5	29.2	0.32	26.0	92	52	0.31	63
15.3	36.6	29.1	0.32	26.6	140	29	0.57	50
14.8	35.3	29.2	0.21	24.3	110	27	0.50	36
14.6	36.4	28.6	0.41	24.5	85	53	0.76	71
16.0	36.0	28.9	0.22	25.1	120	32	0.36	42
16.4	35.7	28.9	0.29	25.2	119	35	0.55	52

Table 2. Average values of mean arterial blood pressure and heart rate of nine curarized hypothermic dogs.

Item	Before curare	After curare	Before cold	Out of bath (28.9°C av.)	Lowest temp. (25.2°C av.)	30°C
Mean BP, mm-Hg	137	54	129	116	108	125
HR, beats/min	148	110	138	87	67	99

warming before shivering occurred or artificial rewarming was initiated.

The experiments reported here (*8*) show that body temperature may be restored to or toward normal following acute exposure to cold without increased striated muscle activity, and they support the hypothesis that heat-producing mechanisms other than muscular activity exist which regulate body temperature.

In order to insure complete inactivity of striated muscle, all animals in our experimental series were heavily curarized to the point of complete respiratory arrest and were maintained at this level throughout the experiments. The experimental procedure was as follows. Short-lasting anesthesia was obtained initially by giving 35 mg/kg of sodium pentothal intravenously. The trachea and appropriate blood vessels were cannulated, and the threshold stimulus of a motor nerve was determined. Ten LD of *d*-tubocurarine chloride (2 mg/kg) (*9*) was then administered intravenously, and artificial respiration was instituted at once; this was followed by 1.8 LD of *d*-tubocurarine every hour until the dog was sacrificed. At various intervals curarization was verified by demonstrating the inability of the stimulated motor nerve to cause muscular contraction.

Since blood pressure falls following curarization (*10*), time was allowed (approximately 1 hour) for the blood pressure to rise to normal before the dog was cooled. Each dog was placed in an ice and water bath (0°C) up to the neck and maintained there until the rectal temperature reached approximately 29°C, at which time the dog was removed, dried, and wrapped loosely in a woolen blanket to minimize heat loss. Animals were observed until the rectal temperature had rewarmed to 30°C or above; all experiments were conducted in a temperature-controlled room at 24°C.

Artificial respiration was given by means of a positive-negative phase pressure pump, because use of the negative phase has been shown to minimize the depression of blood pressure suffered by artificially respired animals in states of mild circulatory embarrassment (*11*). All animals were hyperventilated to reduce the tendency toward cardiac irregularity observed in hypothermic animals (*12*).

Table 1 summarizes data obtained from nine hypothermic curarized dogs. All dogs experienced a drop in rectal temperature of about 4°C after removal from the bath before spontaneous rewarming began. This drop in temperature occurred over a period of approximately 55 minutes. Before sacrifice, one dog rewarmed to 35.5°C, one dog to 34°C, five dogs to 31° to 32°C, and two dogs to 30°C. The rate of rewarming, however, was calculated for the time span between the point of lowest temperature and 30°C.

Table 2 shows that blood pressure was well maintained in all experiments. Cardiac irregularity in the form of a 2 to 1 block was observed as a transient phenomenon at the lowest temperatures in two experiments.

The data presented in this series demonstrate spontaneous rewarming with neither anesthetic depression nor increased muscle activity superimposed. To the best of our knowledge, this type of rewarming has not been previously reported in the literature. Since barbiturate anesthesia is well known to cause marked depression, it may be that the use of barbiturates has prevented others from observing the type of rewarming described here. We postulate that a thermogenic mechanism exists which is not operative when an animal is depressed.

Although Davis and Mayer (*13*) present evidence that in the rat there is a fraction of thermogenesis not due to reflex striated muscle contraction, it should be pointed out that the rats in their series were only partially curarized, whereas the dogs reported on here were completely curarized.

The increased metabolism of visceral tissue measured by You and Sellers (*3*) in cold-acclimatized rats might also occur in hypothermic animals to raise the body temperature. It is further possible that a factor produced in muscle may stimulate liver metabolism during hypothermia, as it does during normothermia (*14*). It seems unlikely that the rewarming process we describe can be attributed to the continuing release of epinephrine, since the period during which rewarming takes place is prolonged (about 9 hours from the lowest temperature to 30°C) (*15*).

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15. The opinions and assertions contained herein are the private ones of the writers and are not to be construed as official or as reflecting the views of the Navy Department or the naval service at large.

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Contamination of Human Cell Cultures by Pleuropneumonialike Organisms

In the course of a study to determine the effect of pleuropneumonialike organisms of human and animal origin on various types of cell cultures available in the laboratories of the department of microbiology, School of Hygiene and Public Health, Johns Hopkins University, a human strain of pleuropneumonialike organisms was inoculated into tubes of HeLa cells. After 48 hours of incubation the fluid medium was removed from the inoculated and the uninoculated control HeLa cell cultures, and 0.1 ml of each was planted onto plates of pancreatic digest agar, using the method previously described for cultivation of pleuropneumonialike organisms (*1*). Growth of typical colonies of pleuropneumonialike organisms was noted on all plates, including the plates inoculated with the fluid from the control HeLa cell culture. This finding was checked and found to be reproducible.

Careful analysis of the experimental design has since shown that the isolated strain of pleuropneumonialike organisms could not have been introduced at the time at which the experiments were carried out. Following this observation, two other sublines of HeLa cell cultures maintained in the same laboratory by other workers, as well as a subline of conjunctival cells maintained in this laboratory, and a culture of HeLa cells recently purchased from a commercial laboratory were examined. The results are summarized in Table 1. It was estimated that there were 1000 to 5000 pleuropneumonialike organisms per milliliter of culture fluid in the positive HeLa cell cultures and 3000 to 10,000 organisms per milliliter in the human conjunctival cell culture (Fig. 1). The organisms from both sources grew well in subculture in liquid as well as on solid media. Growth in liquid media produced no turbidity.

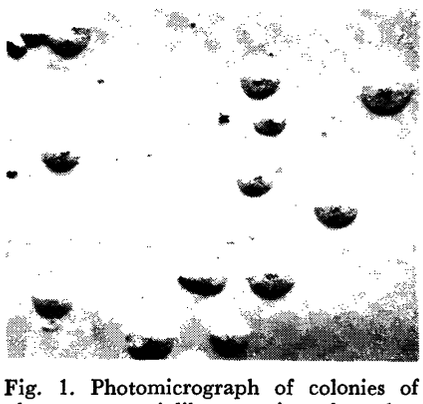

Fig. 1. Photomicrograph of colonies of pleuropneumonialike organisms from human conjunctival cell culture. (× 75)

It appears that sometime between October 1955 and April 1956 the main sublines of cell cultures became contaminated with pleuropneumonialike organisms, probably through some common constituent of culture media. It is of interest that for some time within this period, focal necrotic areas and considerable granulation of cells were frequently manifest.

Contamination of a tissue culture with an unknown agent later identified as pleuropneumonialike organisms has been encountered previously by other workers (*2*). Pleuropneumonialike organisms may easily escape detection or recognition unless they are specifically looked for. Their colonies are microscopic. In liquid media many strains, particularly those of human origin, produce either no turbidity or turbidity which is so slight that it is not readily apparent to the naked eye. They are filtrable microorganisms (*3*) and, therefore, are not removed by most routine filtration procedures used in the preparation of tissue-culture media. They survive freezing. Penicillin in concentrations customarily added to tissue-culture media has no effect on them. The effect of streptomycin of similar levels is variable, depending on the sensitivity of individual strains. The majority of strains of pleuropneumonialike organisms isolated from various sources are sensitive to antibiotics of the tetracycline series (*1*). The strains isolated from the HeLa cell cultures and from the subline of conjunctival cells were found to be sensitive to 4 μg oxytetracycline per milliliter and 256 μg streptomycin per milliliter.

Information regarding metabolic activities and nutritional requirements of members of the pleuropneumonia group is still incomplete (*4*). Recently a few data have become available regarding the effect of these organisms on living tissues. Potential cell parasitism and cell damage are suggested by the demonstration of intracytoplasmic inclusions due to pleuropneumonialike organisms (*5*) and by the abnormal appearance of the HeLa cell cultures examined by us. The undetected presence of pleuropneumonialike organisms may, therefore, invalidate or confuse observations made on presumably uncontaminated cultures.

Table 1. Data on cell cultures examined for presence of pleuropneumonialike organisms (*6*).

Cell culture	Growth of pleuropneumonialike organisms	Source	Maintenance
HeLa 1	+	JH*	Routine† by A
HeLa 2	+	JH*	Routine by B
HeLa 3	+	JH*	Routine by C
HeLa 4	0	JH*	Frozen October 1955
HeLa 5	0	CL	Obtained May 1956
Conjunctival	+	JH	Routine‡

* Strain originally obtained from CL (Commercial Laboratory) in summer of 1955, maintained at the department of microbiology, School of Hygiene and Public Health, Johns Hopkins University.
† HeLa cells: 40-percent human serum, 60-percent Hank's saline, penicillin 100 units/ml, streptomycin 100 μg/ml.
‡ Conjunctival cells: 10-percent human serum, 90-percent Eagle's medium, penicillin 100 units/ml, streptomycin 100 μg/ml.

The findings presented here indicate that tissue cultures or the components of tissue-culture media may be contaminated with pleuropneumonialike organisms and that this contamination is not readily apparent. Routine or at least frequent checks of the cultures and media for these organisms should be considered. In addition, the incorporation of a suitable level of one of the antibiotics of the tetracycline series may aid in the prevention of contamination by pleuropneumonialike organisms.

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