Table 3. Sequences that account for all of the nucleotide residues in the alanine RNA.

pG-G-G-C-, G-U-G-, U-MeG-G-C-, G-C-, G-U-A-G-, DiHU-C-G-, G-DiHU-A-G-, C-G-, C-DiMeG-, C-U-C-C-C-U-U-I-G-C-, MeI-ψ-, G-G-G-A-G-A-G-U*-C-U-C-C-G-, G-T-ψ-C-G-, A-U-U-C-C-G-, G-A-C-U-C-G-, U-C-C-A-C-C-AOH

tion of the dihydrouridylic acid residues may be to "insulate" the coding triplet from the influence of neighboring nucleotides in the polynucleotide chain. Another possibility for the coding triplet is the I-G-C sequence, which is in the middle of the chain and is particularly sensitive to ribonuclease T1. This sequence would be expected to be equivalent to G-G-C in coding properties. The sequence that is the actual coding triplet is still unknown.

It is of interest to consider the structure from the standpoint of the conformation of the RNA in solution. Examination of the nucleotide sequence shows that there are no long complementary sequences that could give G paired with C and A paired with U. In fact, the longest complementary sequences contain only five nucleotides. As a consequence, double-stranded regions must be relatively short or must contain many imperfections in base pairing. Three conformations that utilize different regions of the RNA chain in Watson-Crick type base pairing are shown schematically in Fig. 2. These should be considered only speculative, and in any case it is likely that the actual conformation of the RNA in solution varies with the conditions. The factors that might be expected to influence the conformation of an RNA have been discussed (*12*).

The determination of this structure of a nucleic acid represents the successful conclusion of a major undertaking—an attempt to isolate a biologically active nucleic acid and establish its structure. At the time that the isolation of the alanine RNA was undertaken, it was not known whether an individual nucleic acid could be isolated from a complex mixture of nucleic acids. Once individual transfer RNA's were isolated, it was not known whether a nucleic acid structure could be determined. In retrospect, the part of the structure determination that was completely uncharted, namely the determination of long nucleotide sequences, turned out to be easier than was anticipated. The time-consuming part of our work was the identification of new nucleotides and the proof of structure of the small fragments obtained by complete digestion of the RNA with pancreatic ribonuclease and ribonuclease T1. Once the analyses of the two digests were finished, the elucidation of the structure was greatly facilitated by two experimental developments. The first was the discovery that ribonuclease T1 shows a high selectivity of action at 0°C, making it possible to isolate large fragments from the RNA molecule. The second was the development of highly efficient chromatographic methods which make use of 7*M* urea solutions (*13*) with long, narrow DEAE-cellulose columns and which provide the resolution required to separate small amounts of many different oligonucleotides (*8, 9*). Together, these developments made it possible to isolate and analyze large fragments of the RNA, and the results furnished sufficient information to establish the sequence.

Determination of the structure of the alanine RNA indicates that the structures of other nucleic acids can also be determined and provides a basis for attempts to synthesize a biologically active nucleic acid.

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4. Abbreviations: p and - are used interchangeably to represent a phosphate residue; A-, adenosine 3′-phosphate; C-, cytidine 3′-phosphate; COH, cytidine (with the free 3′-hydroxyl group emphasized); DiHU-, 5,6-dihydrouridine 3′-phosphate; DiMeG-, N^2-dimethylguanosine 3′-phosphate; I-, inosine 3′-phosphate; MeG-, 1-methylguanosine 3′-phosphate; MeI-, 1-methylinosin 3′-phosphate; ψ-, pseudouridine 3′-phosphate; T-, ribothymidine 3′-phosphate; U-, uridine 3′-phosphate; U*-, a mixture of U-, and DiHU-; p!, 2′,3′-cyclic phosphate, for example: Ip!, inosine 2′,3′-cyclic phosphate; DEAE, diethylaminoethyl.
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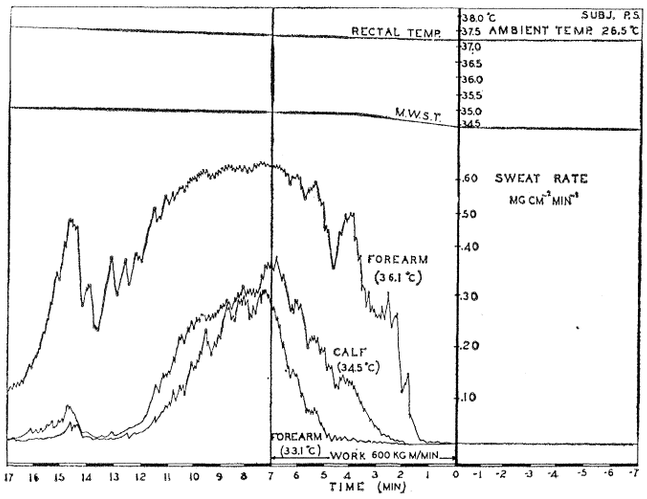
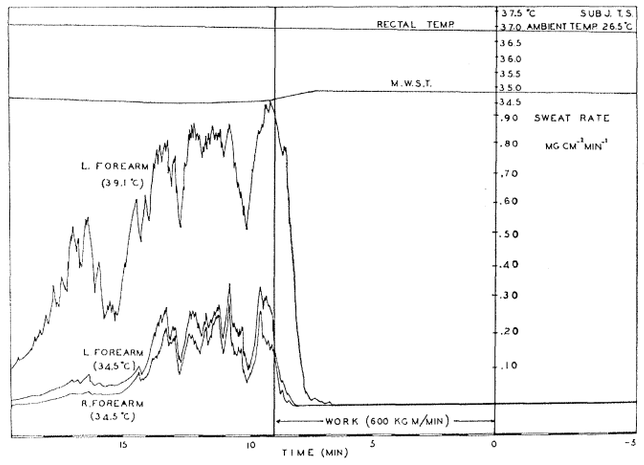
Sweating: Direct Influence of Skin Temperature

Abstract. *The onset and magnitude of the sweating response is greatly influenced by the local skin temperature. This influence was evident with skin temperatures above 33°C and occurred in the physiological range for thermo-regulatory sweating. The sweating rates were recorded continuously and simultaneously from three areas of the skin.*

In spite of extensive study a universally accepted theory of the regulation of thermal sweating in man has not been developed. It has been suggested that the regulation of body temperature depends upon a proportional control system, in which the magnitude of the response is a function of an error signal. This signal is the difference between the actual hypothalamic temperature and a set point temperature (*1*). There is, however, a lack of agreement concerning the participation of skin temperature in this regulation. Interdependence of sweating rate and skin temperature had been suggested earlier by the findings of Kuno (*2*), Bazett (*3*), and Randall (*4*). From experiments in which rates of work and metabolism and rectal temperatures were maintained constant, Robinson (*5*) concluded that the rate of sweating was proportional to skin temperatures up to 35.5°C. Recently Belding and Hertig (*6*) in a





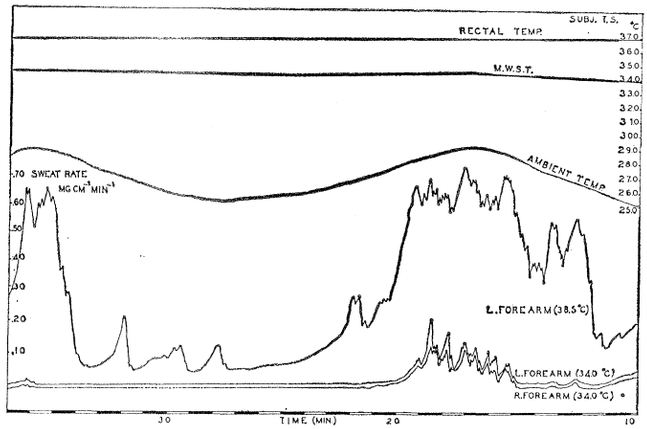


Fig. 1 (top left). Continuous recording of sweat rates from skin areas with different local temperatures with the subject doing moderate physical work on a bicycle ergometer in a cool room. Read from right to left; zero time indicates the start of the work. M.W.S.T., mean weighted skin temperature.

Fig. 2 (top right). Sweat rates from skin areas with a greater range of local temperatures with the subject doing moderate physical work in a cool room. Low internal temperature prolonged the delay of sweating response.

Fig. 3 (bottom left). Differential response from sweat glands in similar skin areas with different local skin temperatures during cyclic variation of the ambient temperature. This recording was obtained from the 10th to the 37th minute of a recovery period after moderate physical exercise in a cool room.

study on working subjects and Wurster, McCook, and Randall (*7*) and Bullard (*8*) in studies on resting subjects have presented evidence that sweating varied with average skin temperatures which were above 33°C. However, Benzinger *et al.* (*9*) have suggested that in a warm environment the rate of sweating is related only to the hypothalamic temperature and is not influenced by skin temperatures in the range of 33° to 39°C.

In our study a very definite effect of skin temperature on sweating is demonstrated by a comparison of the rates of secretion at selected areas of the skin having different temperatures, but receiving equivalent efferent drives from the central nervous system. The rate of sweating was continuously and simultaneously recorded from these areas with a three-channel resistance hygrometry system (*10*). In this method, air of fixed relative humidity was passed through capsules enclosing 12 cm^2 of skin, and the changes in relative humidity of the effluent air were then recorded. The skin temperature under one of these capsules could be increased and maintained at any desired temperature by a small-resistance heating coil mounted within the capsule and controlled with a transformer.

The record (Fig. 1, which reads from right to left) indicates that during moderate work on a bicycle ergometer, in a cool environment, sweating was much greater in the locally heated skin area. During the control period (minute −7 to 0) no sweating was seen in any of the three areas. With the initiation of the work period the sweat glands of the forearm area at a temperature of 36.1°C responded earlier and more intensely than those of cooler areas. A second record (Fig. 2) shows that this differential effect was intensified with a higher local skin temperature (forearm 39.1°C). These responses have been consistent in 15 experiments on four male subjects and indicate that the rate of sweating is strongly influenced by the skin temperature of that area.

In an earlier report (*11*), we presented evidence that maximum work in a warm room produced an increase in sweating within 1 to 2 seconds. In a cool room this rapid response could be evoked only in the locally heated skin area.

Similar effects were also demonstrable in resting subjects. The recording in Fig. 3 was taken during the recovery period after moderate exercise in a cool environment, where the room temperature cycled between 25° and 29°C. Thus, the sweat glands of the warmed skin were affected to a greater extent by variation of the temperature of the environment than those of the cooler areas, even though the warmed skin was not directly exposed to the room air. While it is possible that the hypothalamic temperature may have been varying, the response of the effector system here was greatly enhanced by an increased local skin temperature in the three types of experiments.

The overall results indicate that the temperature of the human skin has an important local influence on the activ-

ity of the eccrine sweat glands. The nature of the mechanism is largely unknown. The effect seems to be of a permissive or facilitatory nature such that the action of efferent impulses upon the sweat glands is enhanced. There may be two explanations: (i) The local excitatory state of sweat glands is increased by heat and decreased by cold directly or through a type of axon reflex. (ii) The facilitation occurs by the interaction of impulses from heat receptors and efferent sympathetic fibers by some unknown mechanism.

Lloyd (*12*), studying the influence of temperature upon the response of sweat glands in the footpad of the cat, found a shorter latency of sweat emergence with higher local temperature; he concluded that for each nerve impulse the glands yield an amount of sweat proportional to the local temperature.

In many studies the influence of skin temperature on sweating was investigated by changing the temperature of large areas of skin or that of the entire surface; such a change, however, influences the mean body temperature and presumably the hypothalamic temperature. Thus, separation of surface effects and central regulation has been difficult. We differ with Benzinger *et al.* (*9*) in that our results indicate that skin temperatures over 33°C have a marked effect on sweating. Their suggestion that the inhibitory effect of cooling is produced only by impulses from cold receptors inhibiting the efferent outflow of the hypothalamus should therefore be reconsidered.

The local thermal effect on sweat gland activity appears to be an important factor in thermoregulatory control. The magnitude and response time of the immediate changes in sweating with variations in mean skin temperature (*6, 7*) must have been influenced by the local excitatory state of the sweat glands.

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Hepatic Glycogen Depletion in Amphiuma during Induced Anoxia

Abstract. *Giant salamanders,* Amphiuma means, *measuring 240 to 280 millimeters from snout to vent, tolerate induced anoxia for 6 hours. After 3 hours of anoxia, hepatic glycogen units are reduced in size and concentration; after 6 hours the glycogen units are almost completely depleted. Greater development and changes in the density of the endoplasmic reticulum indicate that this structure participates in the mobilization of glycogen from the cell.*

Recent studies of the respiratory metabolism of the giant salamander, *Amphiuma means tridactylum*, indicated that these animals could tolerate anoxia for periods up to 13 hours at 22°C. It therefore appeared that *Amphiuma* would be a suitable animal in which to study depletion of hepatic glycogen with the aid of the electron microscope.

Nine animals were placed individually in a nitrogen chamber filled with oxygen-free water. A continuous flow of nitrogen forced the water out of the test chamber and flushed out exhaled oxygen. The chamber was placed in a water bath at 22°C.

Since there was a definite correlation between size and ability to tolerate anoxia in this species, only animals measuring between 240 and 280 mm from snout to vent were used. *Amphiuma* within this size limit tolerated anoxia for about 6 hours. Animals were removed after 3 and 6 hours of anoxia, their body cavities opened, and comparable portions of liver removed. The tissue was fixed in 2-percent osmium tetroxide buffered in Millonig's phosphate buffer (*p*H 7.6), embedded in Epon (*1*), sectioned, and stained for 10 minutes in saturated aqueous uranyl acetate and then for 5 minutes in lead citrate (*2*). Animals maintained in the laboratory for 5 days were used as controls.

As aptly reviewed by Revel, two basic types of glycogen can be recognized with the electron microscope (*3*). Alpha units are complex conglomerates of glycogen particles that assume a characteristic rosette-shape. Beta units are smaller and have smoother edges than alpha units, and they may be analogous to the glycogen particles that comprise the alpha units. Hepatic glycogen in amphibians is usually distributed as individual particles (beta units) (*3*), but this was not the case in *Amphiuma*.

Liver cells of the control animals were packed with glycogen rosettes (Fig. 1*A*). The glycogen was stored in the cytoplasm and the smooth endoplasmic reticulum appeared as short, transparent areas (cisternae) within the cells.

In the animals subjected to anoxia for 3 hours there was an obvious decrease in the size and concentration of glycogen rosettes (Fig. 1*B*). Also, the cisternae were considerably lengthened by the union of shorter sections to form anastomosing channels. The edges of the rosettes were smoother than in the controls, suggesting that the most externally exposed portions had dissolved, leaving a smaller, more rounded unit. At this stage the endoplasmic reticulum appeared more dense than the cytoplasm.

Liver cells of the animals subjected to anoxia for 6 hours were almost completely devoid of glycogen (Fig. 1*C*) and further anastomosing of the endoplasmic reticulum had occurred.

At higher magnifications the glycogen particles of the rosettes appeared to be composed of still smaller, punctate particles (inserts, Fig. 1). These smaller subunits may be either normal facets of glycogen particles, or the effects of nonspecific areas of lead deposition; alternatively, they may be caused by electron-beam bombardment of the specimen (*3*).

There was definite decrease in the number of visible subunits per rosette with increased time of anoxia. Thus, ten of the largest glycogen units had