tigated. Furthermore, reactions overlay cytoplasm and nucleus.

Photographic grains were counted over cellular constituents within areas delimited by either one or three small squares of the Whipple ocular micrometer; in the optical system used these areas measured 4 to 12 μ^2, respectively. The 4-μ^2 area was positioned over the central portion of nucleoli (2 to 3 μ in diameter) in large neurones, liver cells, and spermatocytes; the 12-μ^2 area was employed for the rest of the nucleus and for cytoplasm, and the background grain count (averaging 0.03 per 4 μ^2) was subtracted.

Each recorded figure (Table 1) is the mean of 20 corrected counts (ten cells in each of two animals). The results (Table 1) revealed that radioactivity appeared in the cytoplasm and nucleus of pyramidal cells soon after injection and eventually decreased with time, but was still present at 45 days. The rates of uptake and decrease gave no indication of an interrelationship between nucleus and cytoplasm; both seemed to incorporate amino acids simultaneously and independently. The other cells investigated also showed radioactivity in nucleus and cytoplasm. In contrast, the nucleolus was negative in most cells (Table 1). The few recorded counts may be due to a low degree of amino-acid incorporation into the nucleolus or, since the grains were often located at the periphery of the nucleolus, to uptake by the perinucleolar structures.

The nuclear radioactivity was analyzed by means of drawings of radioautographs of Purkinje cells in which chromatin masses were distinct. In six such drawings, at 35 hours after leucine-H^3 injection, no grains were found over the nucleolus; the grain counts over cytoplasm, nuclear sap, and chromatin were 1.0, 0.1, and 1.6 per 4 μ^2, respectively (the areas were measured by the "paper cut-out" method). Thus, maximal concentration occurred over chromatin. Indeed, grains overlay scattered chromatin masses as well as the nucleolus-associated chromatin. Similar observations at other time intervals and in other cell types confirmed the finding that most, if not all, nuclear radioactivity is located within chromatin masses.

Since chromatin masses consist chiefly of DNA and protein, associated to form chromosomes, the results demonstrated the uptake of three amino acids by chromosomal protein. Similarly, chemical studies by Allfrey *et al.* traced labeled amino acids into all protein fractions obtainable from nuclei, with a maximum in "residual proteins," which are believed to be the protein moiety of chromosomes (*6*). Furthermore, the finding of radioautographic reactions over chromatin material in all animals soon after injection (Table 1) indicated continuous uptake of amino acids by chromosomal protein, while the eventual decrease of the reactions with time (Table 1) indicated turnover.

Theoretically, the incorporation of amino acids into chromosomal protein may be due to adsorption, exchange, or synthesis (which may be total or partial). The maintenance of a reaction over a 45-day period would seem to eliminate the first possibility. The similarity of behavior of all three amino acids is believed to render the "exchange" possibility unlikely. Hence, the amino acids incorporated by cytoplasm and nucleus are believed to have been used for synthesis of local proteins. Therefore, the degree of uptake of leucine, methionine, and glycine reveals that little protein is synthesized from these amino acids in the nucleolus but that active protein synthesis occurs continuously and independently within cytoplasm and nuclear chromatin.

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Effect of Temperature on Isolated Stretch-Receptor Organ of the Crayfish

Abstract. Abdominal stretch-receptor organs of *Astacus leptodactylus* are investigated by means of extra- and intracellular leads. The effects of changing temperature on steady-state rate of activity are surprisingly low. The sensory nerve cell compensates for changes in temperature by means of opposite reactions of generator potential and threshold.

Kerkut and Taylor (*1*), investigating the spontaneous activity of isolated ganglia of the slug, cockroach, and crayfish, found an "anomalous" transient response to changes in temperature. When temperature is increased there is a transient decrease in rate of activity, whereas if temperature is decreased, the activity shows a transient increase. The final rate of activity shows the normal temperature effect: the rate is faster at higher temperatures. The same type of response was reported by Florey (*2*) for stretch-receptor organs in crayfish. This response has now been more closely examined by means of intracellular leads, leading off from the sensory nerve cell.

The time course of spike frequency during and after sudden changes of temperature resembles very much that reported by Kerkut and Taylor for abdominal ganglia of crayfish. The temperature coefficient of activity depends on the stretch to which the receptor is subjected, but in general it is surprisingly low. The receptor may be stretched an additional 100 percent of its length in the relaxed state before impulses drop out [a state known as overstretch (see *3*)]. In the lower part of this working range the rate of impulses in the steady state is nearly independent of temperature, though immediately after a change in temperature a transient, anomalous response is seen. With the increasing length of receptors the Q_{10} increases; values of $Q_{10} = 1.5$ are common. Strongly stretched receptors, nearly in the state of overstretch, show higher values, but in no case has a Q_{10} above 2 been observed. Receptors that are completely relaxed but still spontaneously firing, or very gently stretched receptors, exhibit a Q_{10} below 1; here the steady-state rate of activity decreases with increasing temperature, as in the transient response.

It is thought that the surprisingly low values of Q_{10} and the diphasic time course of activity are both signs of an effect compensating for changes of excitability during changes of temperature. Experiments with intracellular leads have shown that with decreasing temperature the generator potential mediated by stretch increases. This means that the membrane potential attained after a stretch decreases with decreasing temperature. On the other hand, with decreasing temperature a decrease in the critical firing level of the membrane is observed. It is thought (see *4*) that the difference between depolarization of the membrane by generator action and the critical depolarization for spike activity determines spike frequency. This difference remains nearly independent of temperature, in the way mentioned above. The changes of generator potential and threshold after a sudden change in temperature are slightly different with respect to time course; this difference is responsible for the diphasic time course of spike frequency.

An interesting feature is the blockage of impulses by means of extreme temperatures. In the regular pattern of spikes, one suddenly drops out. In the regular place of a dropped-out full

spike, a miniature potential remains. This suggests that rhythmical spike activity is triggered by rhythmical local events in the dendrites or soma of this nerve cell (see *5*).

It seems to be of general interest that the time courses of activity in peripheral nerve cells and in central nervous tissue are similar after a change in temperature. This, together with the finding that the isolated nerve cell already is stabilized against changes of excitability by means of two interacting processes of opposite sign (see *6*), shows that there is a strong compensation for effects of temperature in cold-blooded animals (*7*).

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Second Spectroscopically Abnormal Methemoglobin Associated with Hereditary Cyanosis

Abstract. Isolation of an abnormal methemoglobin from two families exhibiting dominantly transmitted cyanosis have permitted the recognition of two different pigments of the hemoglobin M type. It is possible that the abnormal properties which characterize the acidic methemoglobin derivatives result from a crevice configuration of the heme, with two Fe-protein bonds.

In a previous article (*1*) an account was given of the electrophoretic isolation of both a normal and an abnormal hemoglobin from an affected member of a family exhibiting dominantly transmitted cyanosis. The abnormal component was designated hemoglobin M because the absorption spectrum of the acidic methemoglobin showed the same anomalous features as the oxidized whole hemolysates from the patients examined by Hörlein and Weber (*2*), by Kiese, Kurz, and Schneider (*3*) and by Heck and Wolf (*4*). A second family, living in Canada, with a comparable genetic transmission of cyanosis (*5*) has now been studied, and the presence of a similar spectroscopically abnormal methemoglobin has been demonstrated (*6*). However, upon isolation the latter methemoglobin was found to differ from that present in the first family in several fundamental respects. In the following brief account, the clinical, spectroscopic and chemical features characterizing and distinguishing these two abnormal hemoglobins will be given. To facilitate discussion, the pigment described previously (*1*) will be identified as hemoglobin M, Boston type (symbolized as Hgb M_B), and that described here for the first time will be identified as hemoglobin M, Saskatoon type (Hgb M_S).

Clinically, the presence of either variety of hemoglobin M is accompanied by cyanosis. With Hgb M_B, an increased level of methemoglobin could not be demonstrated by the method of Evelyn and Malloy (*7*). The cyanosis occurring with Hgb M_S, on the other hand, was associated with an increased amount of methemoglobin as determined by this technique (*8*).

Separation of the hemoglobin into two fractions was effected in both types of patients by starch block electrophoresis of the hemolysates after conversion into methemoglobin by treatment with potassium ferricyanide (Fig. 1). Optimum resolution occurred under the conditions of cathodic migration (sodium phosphate buffer, *p*H 7.0, ionic strength 0.1). Each electrophoretic band was distinctively colored: methemoglobin A was brown, M_B was gray, and M_S was green. The several pigments were recovered in pure form by elution after careful excision of each colored band from the starch block. The eluates were then examined spectroscopically by one of us (P. G.).

The absorption spectra of the acidic forms of methemoglobins M_B and M_S from 450 to 700 mμ are shown in Fig. 2 (*B* and *C*). In both, the 632 mμ peak of acidic methemoglobin A (see Fig. 2*A*) is absent, and both are characterized by a new peak at 602 mμ. The intensity of this new maximum relative to the common maximum near 500 mμ is appreciably greater for methemoglobin M_S than for methemoglobin M_B, namely 0.72 compared with 0.61. Furthermore, in the spectrum of methemoglobin M_S there is a poorly resolved band at about 540 mμ which is scarcely perceptible in methemoglobin M_B. This same band is a little more pronounced in methemoglobin A. In the Soret region the maxima for acidic methemoglobin M_B and M_S are at about 406 mμ (like that of methemoglobin A), but both have lower intensities. Relative to the intensity of the Soret band for the corresponding carbonmonoxyhemoglobins, the values are 0.92 for Hgb A, 0.76 for Hgb M_S and 0.62 for Hgb M_B.

In the reactivity of their hemes there is an even more marked contrast: methemoglobin M_S resembles methemoglobin A, whereas methemoglobin M_B is in a class apart.

Methemoglobin M_S reacts rapidly with the ligands F^-, CN^- and N_3^- to give the usual complexes. It is reduced very rapidly by $Na_2S_2O_4$ and is oxidized by H_2O_2 to the higher oxidation state. All these reactions proceed smoothly to completion, and in this respect methemoglobin M_S is indistinguishable from methemoglobin A, although there may be differences between the rate constants which would become apparent in a detailed kinetic study. In addition, although acidic methemoglobin M_S has its own characteristic spectrum, in solutions of *p*H > 9 an alkaline form predominates

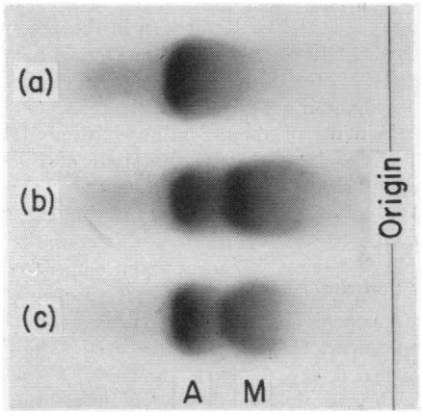

Fig. 1. Starch block electrophoresis of oxidized hemolysates (that is, the methemoglobins) at *p*H 7.0: *a*, normal blood; *b*, Hgb M_S trait; *c*, Hgb M_B trait. Migration is toward the cathode.

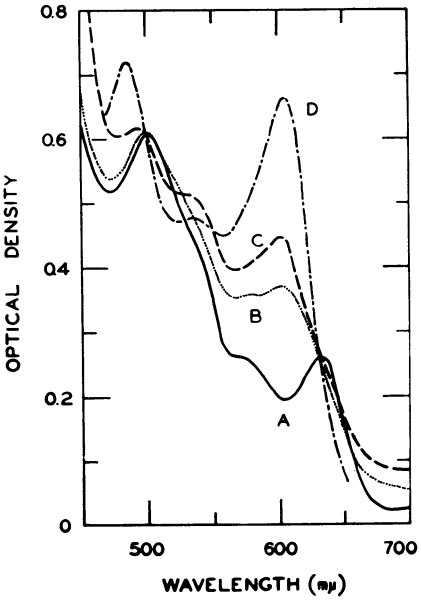

Fig. 2. Absorption Spectra at *p*H 7.0: *A*, methemoglobin A; *B*, methemoglobin M_B; *C*, methemoglobin M_S; *D*, methemoglobin A fluoride complex. For purposes of comparison all the optical densities have been made equal to 0.61 at 500 mμ.