PN 1218

The multiple hemoglobins of some members of the Salmonidae family

The discovery of an abnormal hemoglobin (Hb-S) in the erythrocytes of humans suffering from sickle-cell anemia[1,2] provided the first positive evidence of the existence of more than one molecular form of hemoglobin. Since then many genetically distinct conditions manifesting themselves partly by differences in hemoglobins have become evident[3]. Varying degrees of hemoglobin multiplicities were found in amphibian and reptiles[4] and in fishes by agar-gel[5–7], paper[8], starch-block and free-boundary[9,10] electrophoretic methods, as well as by column chromatographic techniques[11]. Where the same species of fishes, particularly some members of the salmonoid family, have been studied by various investigators and by different methods, up to three hemoglobin components have been reported. In the present study the hemoglobins of some of the same group of fishes were separated by starch-gel electrophoresis, revealing an unexpectedly greater degree of multiplicity.

Blood samples were obtained either by severing the tail peduncle of the fishes or through their cuvierian duct[12] and the sera removed by centrifugation. The red cells were washed three times with physiological saline, lysed with distilled water, and the stroma centrifuged off. Then the freshly prepared hemoglobin was separated by starch-gel electrophoresis by a method previously described[13] at pH's of 8.0 and 8.5.

A simultaneous run at pH 8.0 of human, sockeye (*Oncorhynchus nerka*) and rainbow trout (*Salmo gairdnerii gairdnerii*) is shown in Fig. 1. The gel was stained with amido black 10 B. Normal adult-human hemoglobin, shows the predominance of Hb-Ao and a few minor fractions in agreement with the results of HUISMAN[14] using the same method. In this case the starch gel does not introduce factors leading to the formation of additional hemoglobin zones. The hemoglobins from the sockeye salmon and the rainbow trout, on the other hand, separated into 12–16 zones in contrast to the 3 components reported and also show a wide range of mobilities at this pH and is distributed on either side of the origin. The overall hemoglobin pattern between the two fishes are similar but distinctive species differences are evident. The spring salmon (*Oncorhynchus tshawytscha*) though not shown here also showed the

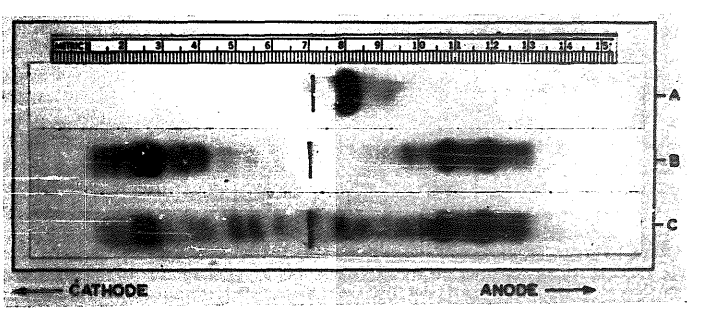

Fig. 1. Comparison of human (A), sockeye salmon (B) and rainbow trout (C), Hb's by starch-gel electrophoresis at pH 8.0 borate buffer.

extreme complexity in its hemoglobin pattern, a property which appears to be shared by the salmonoid fishes in general.

The starch gel separating the hemoglobins of the rainbow trout and the eastern-brook trout (*Salvelinus fontinalis*) was sliced horizontally into three sections and the natural red color of the hemoglobin was compared to the sections stained with the non-specific stain amido black 10 B as well as by the more specific benzidine stain (Fig. 2). The hemoglobin zones from all three sections correspond to each other. The benzidine stain is considerably stronger and more diffuse on the cathode side compared to the other two sections. It is also evident that the rainbow and eastern-brook trouts show distinctive differences in their hemoglobin patterns.

The hemoglobin preparation from the eastern-brook trout was placed in 4 slots and separated simultaneously with the run indicated in Fig. 2. The zones corresponding to No. 1–15 (Fig. 2) were cut out and eluted by a method to be described elsewhere. The absorption spectra of all the zones were similar to HbO_2 of some mammals[15], with maxima at 576 and 540 mμ and minima at 560 and 508 mμ. These results are in agreement with those of HASHIMOTO AND MATSUURA[10] who reported that chum salmon (*Oncorhynchus keta*) hemoglobin displayed visible absorption spectra similar to that of horse hemoglobin. Calculation of percentages of HbO_2 in the presence of hemoglobin and ferrihemoglobin by the method of MEYERING *et al.*[16] showed that the HbO_2 predominated. From these figures it is not very likely that reduced forms of HbO_2 are contributing to the various zones observed. The possibility that some of these zones may be aggregates has not yet been completely ruled out for ultracentrifugal studies, which indicated a uniformity in molecular weights were carried

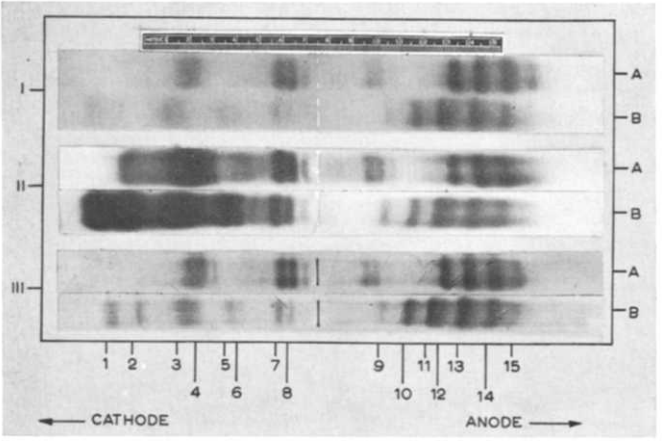

Fig. 2. The starch gel separating the Hb's of rainbow (A) and eastern-brook (B) trouts are sliced into three sections and the Hb red color (I) of one of the sections is compared to the other sections stained with benzidine (II) and amido black 10 B. Electrophoresis was carried out in borate buffer at pH 8.5. The numbers 1–15 refer to the Hb zones of eastern-brook trout eluted for spectral measurements.

out only on sockeye salmon prior to gel separation. However, human hemoglobin under the same conditions showed the normal components. These examples of extreme hemoglobin heterogeneity represent studies carried out on many individuals and pooled blood samples from the same stocks of fishes and probably indicate a pattern present in normal fishes.

The Fisheries Research Board of Canada, Technological Station, Vancouver, B.C. (Canada) H. TSUYUKI
R. E. A. GADD

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Temperature-induced changes in phosphorus metabolism in synchronized Tetrahymena

Continuing their studies on phosphorus metabolism in dividing cells, the authors have demonstrated several striking changes in the chromatographic patterns of the trichloroacetic acid-soluble fractions isolated from synchronously dividing *Tetrahymena pyriformis* GL. Following isolation and concentration of the trichloroacetic acid-soluble fractions, paper chromatographic separation of the compounds was achieved using EBEL's solvent system[1]. The locations of the labeled compounds were permanently recorded on radioautographs. By rigidly controlling the labeling, isolation and chromatographic procedures, very reproducible results and a high degree of resolution were obtained.

Using these methods, the authors were able to demonstrate that certain compounds accumulated at one stage or another. Qualitative alterations were observed between the stages; some of the compounds appeared (at the experimental level of identification) to be completely absent. In addition to non-dividing and synchronously dividing cells, normal logarithmic and stationary-phase cells were included as controls and for comparison.