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.

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PN 1224

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.

The growth conditions of the synchrony-inducing heat treatment have been described at length in a previous paper[2]. In the present series of experiments, the samples were removed from the culture at the five growth stages mentioned in the legend to Fig. 1. The size of the samples varied from 470 ml to 80 ml as the concentration of the cellular material increased. The cells were collected by centrifugation, washed in 0.4 % NaCl and extracted 3 times for 1 h each time with 15 % trichloroacetic acid in an ice bath. Glycogen was removed by addition of an equal volume of 95 % ethanol. After removal of trichloroacetic acid by ether, the extract was concentrated by lyophilization to a volume of about 0.5 ml.

After the determination of total phosphorus using the method of FISKE AND SUBBAROW[3], the remaining extract was chromatographed according to the two-dimensional procedure established by EBEL[1]. Following chromatography, radioautographs were made to determine the positions of the labeled phosphorus compounds. Repeated experimentation showed the results of this procedure to be highly consistent.

The distribution of the labeled compounds isolated in the trichloroacetic acid-soluble fraction is shown in Fig. 1. The number of definable spots varied from stage to stage, and only the easily seen spots are numbered on the chromatogram. Using larger amounts of extract, more spots could be obtained, but resolution was poor. The most significant changes occurred in the compounds numbered 17, 18 and 19. These compounds, which were barely discernible during untreated exponential multiplication, increased during synchronization treatment until the dense amounts seen in stage 2 (end of the heat treatment) had accumulated. After the heat treatment, when the cells are going through the cytological recovery phase and preparing for division, the amounts of these compounds gradually diminished. Relatively large amounts still remained 20 min prior to the first synchronous division (stage 3), but 1 h after the first synchronous division, these compounds had decreased appreciably (stage 3'). In the stationary phase, collected at least 18 h later, the level of these compounds was also greatly reduced. This pattern suggests that the accumulant is a normal pathway product rather than one toxic to division, since division occurs in the presence of relatively large amounts. The relation to nucleoside triphosphate production noted previously[4,5] is of interest.

Unlike the gross alterations appearing in compounds 17, 18 and 19, other, and somewhat less marked, variations can be seen in the region of compounds 20 through 24. With the possible exception of spots 21 and 24, there is no apparent relationship between the behavior of one spot and that of another. Similarly, changes which seem closely related to cessation of division in the stationary phase do not occur or are reversed in cells which cannot enter division due to the heat treatment.

In a preliminary work such as this, there can be little valid correlation made between the changes observed on a paper chromatogram and those occurring within the cells or its particulate components. However, the accumulation of compounds 17, 18 and 19 suggests that an alteration in the metabolism of low-molecular-weight phosphorus compounds is produced by the synchronizing treatment. Whether or not this accumulation is the source of the cells' inability to divide while undergoing the heat treatment is another question. It should be remembered in this connection that synthetic pathways, which also require energy, are not blocked (the cells undergo an impressive increase in size during this period).

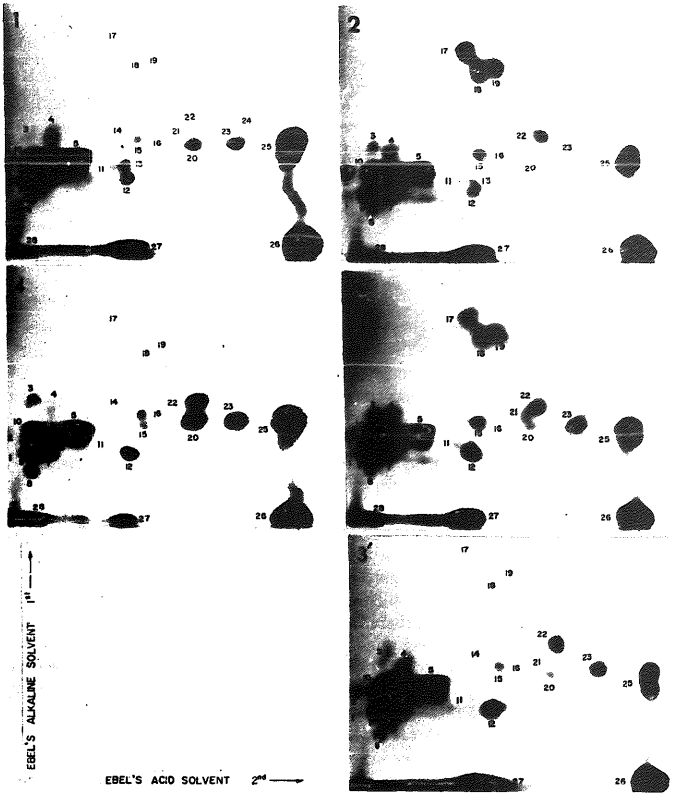

Fig. 1. Radioautographs of two-dimensional paper chromatograms of an acid-soluble fraction of Tetrahymena. Materials used: Filter paper from Schleicher and Schüll, 589 orange ribbon; Kodak no screen X-ray film; 10 μg phosphorus was applied to each paper. Solvents: first dimension, isopropanol–isobutanol–NH_4OH–water (40:20:1:39, v/v); second dimension, isopropanol–trichloroacetic acid–NH_4OH–water (75:5 g:0.3:25). Time of development at 24°: first dimension, 40 h; second dimension, 20 h. The chromatograms were exposed to film for 2 weeks. The numbers in the upper corner refer to the following growth stages: (1) exponential multiplication at 29°; (2) immediately following the seventh and last heat shock at 34°; (3) 20 min prior to the first synchronous division (*i.e.* 1 h after stage 2); (3') 140 min after the end of the heat treatment (interphase between the first two synchronous divisions); and (4) during the maximum stationary phase.

These experiments are being continued in the form of an analysis of the classical pathways of ATP production with concurrent attempts to refine and identify the compounds which have been isolated, particularly those which accumulate.

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Fractional solubilization of haemoproteins and partial purification of carbon monoxide-binding cytochrome from liver microsomes

Recent work in this laboratory[1,2] has demonstrated that the CO-binding pigment discovered in liver microsomes[3,4] is in fact a new cytochrome of the *b* type. This cytochrome shows anomalous spectra in the microsomal bound form, but behaves as a typical haemoprotein when converted to a solubilized form. The microsomal bound and solubilized forms of the pigment have been tentatively called P-450 and P-420, respectively, to distinguish them from each other. This pigment has, however, not yet been separated from cytochrome b_5, another microsomal haemoprotein, and its properties have so far been studied only by means of difference spectrophotometry. This paper reports successful separation of the two microsomal haemoproteins by fractional solubilization and briefly describes partial purification of P-420. The microsomes used were prepared from rabbit liver by a modification[2] of the method of MITOMA *et al.*[5].

As already reported[1,2], anaerobic treatments of microsomes with heated or une heated venom of the snake, *Trimeresurus flavoviridis*[6], or with deoxycholate led to almost quantitative solubilization of both cytochrome b_5 and the CO-binding cytochrome, the latter being fully converted to the form of P-420. As can be seen from Table I, however, digestion of microsomes with crude pancreatic lipase ("steapsin") under suitable conditions resulted in the quantitative solubilization of only cytochrome b_5. Most of the CO-binding cytochrome thereby remained attached to the undigested particles and could be sedimented by centrifugation at 105000 × *g* for 60 min. The sediment, free from cytochrome b_5, represented about half of the microsomal protein and will be referred to as CO-binding particles. In these particles the CO-binding pigment existed both in the forms of P-450 and P-420 as evidenced by the appearance of two absorption maxima at 452 mμ and 421 mμ on addition of CO to dithionite-reduced particles, corresponding to the formation of CO compounds of