in the usual manner (*1, 2, 13*). Trial chromatograms with *n*-butanol disclosed that excellent separation of sugar spots was obtained in 36–48 hr. This time was adjusted so that the most rapidly migrating sugar remained well above the lower end of the chromatogram. The papers irrigated with ethyl acetate were allowed to run for 24–30 hr. After irrigation, the solvent was removed from the chromatograms by evaporation in a circulating air oven at 85° C. The sugar spots were located by spraying with the silver nitrate reagent of Partridge (*2*) and developing in an oven at 85°–95° C.

Since the solvent was allowed to flow off the end of the paper it was not possible to calculate the R_F value (*2*). Instead, the value "R_X" was used. With this system, xylose was arbitrarily assigned a migration distance of 1.00, and the location of the other sugar spots was calculated proportionally.

Two additional solvents were employed in an ascending chromatographic method, the phenol-ammonia mixture of Partridge (*2*) and commercial acetone to which 10% water by volume was added. In these chromatograms, the filter paper sheets were rolled into cylindrical form and allowed to irrigate by the ascending technique of Williams and Kirby (*14*). Five to eight hours were sufficient for migration of the acetone and 10–12 hr for the phenol. Development of the spots was conducted as with the other solvents. R_F values were calculated for these chromatograms by the usual method (*2*).

The separation of the components of the hydrolysates was not equally good with all the solvents. Results with the *n*-butanol and ethyl acetate were more satisfactory than with the other two solvents. This was partly due to the better separation possible with the modified descending technique (*13*) used with the *n*-butanol and ethyl acetate.

An examination of the R_F and R_X values in Table 1 reveals that the polysaccharides of all three types exhibited spots migrating similarly to xylose, mannose, and galactose.

In chromatograms irrigated with butanol, mannose was differentiated from fructose by spraying with a resorcinol-HCl mixture (*15*). The fructose control produced a bright red spot when this reagent was employed, but the mannose spot was barely visible.

Each hydrolysate possessed an additional slower-moving spot in the region of galacturonic acid and glucuronic acid. This spot did not coincide exactly with either of the two reference uronic acids, however, and since additional uronic acids were not available the spot has not been identified. It seems likely that this spot represents the uronic acid responsible for positive Dische tests (*4, 5*). The uronic acid spot was well isolated only with the butanol-acetic acid mixture. It formed a trail when phenol and acetone were employed as solvents.

The hydrolysates of polysaccharides from each of the three antigenic types of *C. neoformans* are qualitatively similar. The fact that all three polysaccharides contain the same four monosaccharides offers some insight into the close antigenic relationships among the three types (*5, 11, 12*).

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The Relationship of Muscle Potassium to the Melanophore-concentrating Effect of Pressure on the Trout[1]

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Observant fishermen have noted that not only do trout lose their brillant coloring at varying intervals of time after being taken from the water but also that the pallor is often patchy and occurs principally at points of pressure. The patterns of leaves or ferns used for packing the creel are frequently sharply outlined on the trout's skin. Fish kept for relatively long periods tend to become uniformly pale. This latter type of pallor is very likely due to the melanophore-concentrating effect of temperatures above those to which the trout is accustomed (*1*). That caused by pressure has not been explained satisfactorily. The only investigator who has apparently paid any attention to this phenomenon is von Frisch (*2*). He concluded that concentration of the melanophores from pressure was due to the local accumulation of acid products of metabolism. The experimental findings outlined in the present communication point to another mechanism.

Observations on the effects of pressure applied to the body surface of the trout under varying conditions have been described in a previous study (*1*). The salient findings were as follows:

1. A rainbow trout (*Salmo gairdnerii*), killed instantly by a sharp blow on the head and laid on a hard surface, exhibited pallor of the underside within 20–30 min. Turning the fish over resulted in a gradual dispersion of the concentrated melanophores, and those of the side now underneath concentrated. This reversal phenomenon, dark to pale and pale to dark, could be induced repeatedly. Pallor from pressure

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was inhibited by lowering the surface temperature sufficiently. The duration of pressure rather than the intensity appeared to be the important factor.

2. Pressure on strips of skin isolated from the body produced no change in the state of dispersion of the melanophores contained therein. However, when pressure was applied to excised blocks of tissue consisting of skin and underlying muscle, concentration of the melanophores occurred just as if the tissue were still a part of the dead fish. This suggested that pressure brought about the transfer of some melanophore-concentrating substance from the muscle or subcutaneous tissues into the skin.

3. Strips of skin removed from the trout and replaced on the subcutaneous tissue showed no effect from applied pressure. The result was quite different, however, when a skin strip was laid on exposed muscle. Marked melanophore concentration and pallor took place within a minute or two. Extracts of the dead trout's muscle made by maceration in 0.9% NaCl solution were equally effective.

4. That pressure-induced pallor was not simply a post-mortem effect was shown by placing a rubber band around the live trout. Its removal after 45 min revealed a band of pallor encircling the fish's body.

5. Evidence that living muscle is capable of liberating a melanophore-concentrating substance was obtained by laying a strip of skin (from another trout) on the exposed muscle of a live trout with its head kept under water during the procedure. Concentration of the melanophores of the skin strip at points of contact with the muscle was just as rapid as when muscle of dead trout was employed.

Of the known substances in muscle that possess the power to produce concentration of melanophores—namely, potassium and calcium—the former exhibits considerably greater activity in this respect (*1*) and is very much more abundant than calcium. Furthermore, since potassium constitutes by far the predominant base of muscle, it is unlikely that any other ion (e. g., magnesium) could be responsible for this effect. Determinations were made of the potassium content of trout muscle (freed from fat) and of extracts (equal parts by weight of muscle and 0.9% NaCl solution, ground in a mortar) in order to ascertain whether the concentrations of potassium present were sufficient to produce the degree of melanophore contraction described. Trout muscle was found to contain 129 mEq potassium/kg muscle, which is a relatively high figure for vertebrate muscle. The muscle extract contained 66 mEq/kg fluid,[2] which in terms of KCl would equal 0.49% solution. It was found in the previous study that 0.8% NaCl was required to neutralize the melanophore-contracting effect of 0.1% KCl. Actual tests with 0.5% KCl showed that even 2% NaCl, which by itself produces a marked dispersion of the melanophores, when added to the KCl solution did not prevent the contracting effect of the potassium. Another pertinent observation of the earlier study was that the potassium ion is able to penetrate rapidly the subcutaneous surface of the isolated skin strip.

Although these findings made it evident that release of potassium from the trout's muscle could account for the above-described effects on the melanophores, they did not exclude the possibility that the contracting agent might be an organic compound. To elucidate this point the following procedures and tests were carried out. Muscle extracts dialyzed through cellophane bags were found to have lost their activity for melanophores. Although this would eliminate organic compounds of large molecular weight, such substances as adrenalin are readily dialyzable (*3*). On the other hand, boiling extracts and keeping them in an unsterile state for 2 weeks did not reduce their potency. As a further, and crucial, procedure a muscle extract was incinerated, water was added, and the H-ion concentration adjusted to bring it back to its original volume and reaction (pH 8.0). This reconstituted extract exhibited fully as great melanophore-contracting activity as did that part of the same extract kept untreated as a control; hence there seems to be no doubt that the active principle is an inorganic compound.

Further studies showed that slices of trout brain, liver, and spleen, tissues known to be relatively rich in potassium, all possessed the property of producing contraction of the melanophores of skin strips. Fresh beef muscle also exhibited the same effect, whereas blood serum, which contains approximately 5 mEq potassium/l, and a ratio of Na to K of about 30 : 1, caused no change in the state of the pigment cells. These findings add support to the probability that the melanophore concentration and attendant pallor of the trout's skin resulting from pressure are due to liberation of potassium from the underlying muscle.

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Fluorometric Determination of Nicotinamide by Use of Synthetic Ion Exchange Resins

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Chaudhuri and Kodicek (*1*) have reported a fluorometric determination of nicotinamide by treatment with cyanogen bromide solution under specified conditions. In that case, interferences of the other fluorescent substances were practically eliminated by the use

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