acids are uncertain. The purpose of the present work to test the truth of the aforementioned assumption as applied to free sugars in lake water (*5*).

Water samples were collected from two lakes in eastern Ontario: Lake Opinicon, located near Chaffey's Lock, and Little Round Lake, located near Maberly. The former is a shallow eutrophic lake with no thermocline and a maximum depth of 10 m. The latter is an oligotrophic lake with a well-developed thermocline and a maximum depth of 16 m. The water samples were collected with a Kemmerer water sampler and filtered through Millipore HA filters to remove the seston (plankton plus detritus).

Filtration was completed within 12 hours of collection for the Little Round Lake samples, and within 30 hours of collection for the Lake Opinicon sample. Before and after filtration the samples were stored at 4°C in the dark. Toluene was added as a preservative after filtration. It was not added before filtration because of the possibility that dying cells might liberate sugars to the water. The filtered samples were deionized with Amberlite IR-120 and IR-4B resins and then concentrated to dryness in a vacuum at temperatures below 45°C.

The dry residues were taken up in 1 ml of water, and amounts ranging from 5 to 300 μlit were spotted on sheets of Whatman No. 1 filter paper. Standard amounts of known sugars were spotted beside the unknowns, and the chromatograms were developed with a butanol-ethanol-water (45/5/50) solvent (*6*) for 3 days by descending chromatography. Chromatograms were run in duplicate. After being dried, one was sprayed with a benzidine spray (*7*) and the other with 2 percent orcinol in 2*N* hydrochloric acid. The amounts of the individual sugars were estimated by comparing the color intensities of standard spots with those of unknowns at the same chromatographic position. The comparisons were made in ultraviolet light. In order to determine the contamination level during analysis three separate samples of double-distilled water were subjected to exactly the same procedure: one 4-lit sample and two 2-lit samples.

The results are presented in Table 1. Sucrose and glucose were the only sugars detected in lake water. Both sugars occurred in low and approximately equimolar amounts. There was evidence in Little Round Lake that increased amounts of free sugars were present in the hypolimnetic water (below 8-m depth). On the other hand, the distilled water controls contained barely detectable amounts of sucrose and no glucose.

Under the conditions used in these analyses, about 25 percent of the sucrose and glucose initially present would have been removed by the ion-exchange columns (*8*). We are not prepared to make any statement about the exact amount of free sugar in the "dissolved" organic matter of lake water but feel justified only in stating that the concentrations are of the order of magnitude of *n* parts per billion parts of water. These low concentrations do not negate the assumption under test.

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Method for Predicting Amount of Strontium-89 in Marine Fishes by External Monitoring

If the total radioactivity in a whole fish could be determined by means of external monitoring, this information would be very useful to ecologists, fisheries biologists, and others. If such a method were found to be suitable for field work, it conceivably could eliminate much tedious dissecting, ashing, and counting procedures. During the course of some experiments on the uptake, accumulation, and loss of radiostrontium by marine fish, a record was kept of the counts per minute detectable at various external parts of the fish's body prior to dissection and analysis of the organs and tissues by conventional ashing and counting techniques. It was not known in advance whether or not these measurements would have any significance, but the possible occurrence of a direct relationship between some aspect of external monitoring and the total amount of radioactivity in the entire fish seemed worth investigating (*1*).

Several species of pelagic fish, including the black skipjack tuna (*Euthynnus yaito*), the yellowfin tuna (*Neothunnus macropterus*), and the dolphin (*Coryphaena hippurus*), were fed radioactive strontium-89 in gelatin capsules. The fish were kept in tanks with circulating sea water and were killed at intervals up to 27 days. A piece of pliofilm (Saran wrap) was used to cover the fish, and the probe of a count rate meter was used to determine the number of counts per minute on direct contact. The G-M tube used had a window thickness of 3.5 mg/cm², but it was protected by a screen that reduced its efficiency to some extent.

The various organ systems and tissues of the fish were subsequently analyzed for radioactivity. The total radioactivity recovered in each system—for example, the gills, axial skeleton, muscle—was then compared with the counts per minute (at 5-percent statistical error) that were previously found by monitoring the mouth, eye, operculum, gills, dorsal integument, and the pectoral, dorsal, and caudal fins. No apparent relationship was observed between any two parameters except that between the amount of radioactivity in the skeleton and the counts per minute found by monitoring the caudal fin. Figure 1 shows that the relationship between the number of microcuries of radiostrontium in the skeleton and the counts per minute in the caudal fin is approximately linear. Since about 30 percent of the radiostrontium found in the fishes after 7 hours occurs in the axial skeleton (*2*), it is therefore possible by measuring the counts per minute in the caudal fin to predict the total amount of radiostrontium in the entire fish.

This method proved successful only when the axial skeleton was found to have a radiostrontium content in excess of an undetermined threshold. Thus, a fish having 0.16 μc Sr^{89} in its skeleton showed only background radiation with the count rate meter, but a fish with 0.40 μc Sr^{89} had sufficient external radiation to be detected. The minimum Sr^{89} skeletal constant is therefore somewhere between

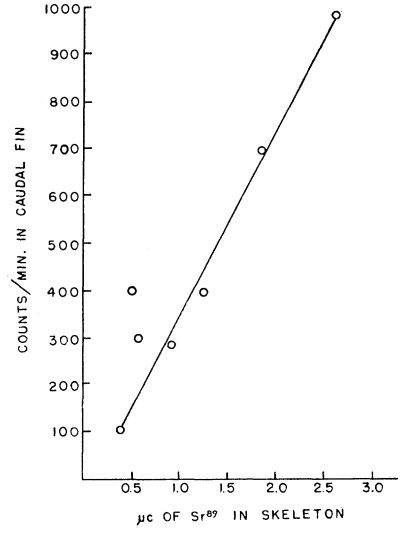

Fig. 1. Linear relationships between the counts per minute monitored in the caudal fin of tuna fed Sr^{89} and the microcuries of Sr^{89} found in the skeleton.

0.16 and 0.40 μc and probably represents the loss owing to self-absorption of the beta-particle energy. These values are, of course, valid only for the particular counting system used in this series of experiments. It is possible to make the detection system considerably more sensitive and, therefore, reduce the threshold value for skeletal strontium.

The afore-described method has not yet been tried with other fission products, and in particular, mixed fission products, such as might be encountered in an area following an atomic detonation or in a region into which nuclear power plant wastes are introduced. If a parameter such as that indicated in this paper could be used to predict the total amount of radioactive material in marine fish, it might be feasible to monitor a catch on the fishing vessel or at the canning factory and, thereby, possibly prevent radioactive fish from being consumed.

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Protein Adsorption on Filter Paper

Protein adsorption on paper is one of the main obstacles in the determination of mobilities by paper electrophoresis, since ion interaction with the supporting medium decreases the migration rates. As surface-active substances, the proteins assemble at the solvent-cellulose interface, and their adsorption behavior will be determined primarily by electrostatic forces between the ion and the negative charge on the paper. The latter would be due to a selective absorption of oxhydrilions from the medium, which accounts for the negative charge on most nonionogenic surfaces in water (*1*). Carboxyl groups that probably originate by an oxidation of primary alcohol groups during the paper manufacture (*2*) may also play a role in this connection. It follows that the paper charge, and the electroosmotic flow arising from the zeta potential thus produced, should increase with the *p*H. In fact, in electrophoretic analyses where dextran was employed to measure the electroosmotic flow (*3*), we found that the migration tended to increase in approximate proportion to the *p*H from *p*H 4.0 to 7.0.

Pretreatment of the paper in order to avoid adsorption has been attempted (*4*) but so far has not met with success. We therefore introduced, among other factors, a correction for this effect in the calculation of electrophoretic migration rates and found complete agreement between the mobilities determined by open-strip paper electrophoresis and those obtained by the Tiselius method (*3*).

Chromatographic R_f values were chosen to evaluate adsorption, since protein migration would not be subject to any forces other than the solvent displacement by capillary action (*5*). Ten microliters of dialyzed solutions of normal human albumin ($5 \times 10^{-4} M$) and gamma globulin ($2 \times 10^{-4} M$) were applied on 3- by 49-cm strips of Munktell paper No. 20/150 2,5 cm above the buffer level, after 18 hours of equilibration (*6*). Sodium and zinc acetate buffers of $\Gamma/2$ 0.045 were used as part of our studies on protein-zinc interactions, and the ascending chromatograms were developed for 120 minutes at 23°C, after which they were stained and scanned, as in electrophoresis. Each of the points recorded represents the mean of four determinations with a maximal variation of about 5 percent.

The R_f minima of albumin (Fig. 1) indicating maximal adsorption at *p*H 4.85 in sodium and at *p*H 6.00 in zinc acetate coincided with the isoelectric points in the same media as found in our electrophoretic analyses. This is in accordance with the early literature on this subject dealing with adsorption on collodium and charcoal (*7*). Gamma globulin, however, showed in sodium acetate a broad R_f minimum constant from *p*H 4.0 to 6.8, the upper limit of which roughly corresponds to the isoelectric point. Analogous results for immune globulin were obtained by Shepard and Tiselius (*8*) from studies on silica gel.

Under the conditions employed, gamma globulin was more strongly adsorbed than albumin in sodium buffer. The difference amounts to 4.5 percent at *p*H 5.0 and increases to above 10 percent toward the extremes of the experimental *p*H range. When, in order to establish conditions prevailing in the whole serum, 1 ml of albumin solution was added per 100 ml of buffer at *p*H 4.05, gamma globulin adsorption fell to the albumin level, evidently owing to a partial neutralization of the paper charges by albumin. The implications of this fact are discussed in another report (*3*).

The fundamental role of electrostatic interactions in adsorption is emphasized by experiments with a practically nonionized substance as dextran. Whereas for a 4-percent aqueous dextran solution we found R_f values of 0.98 ± 0.02 throughout the whole series, Tiselius (*4*) points out that salmine, which mainly bears strongly ionized cationic groups, is so firmly held by paper in electrophoresis that "tailing" impedes the observation of compounds with lower mobilities.

Moreover, when the electrostatic factor is stressed, the results indicate that the protein net charge is of secondary importance in the interaction with paper centers, which will be sustained by the cationic groups of the protein. The reason for the adsorption falling off in strongly acid media will thus be the low charge on the paper, and its decrease above the isoelectric point of the protein will be due to the diminution of the positive protein charge. Hence, adsorption will be maximal in a zone around the isoelectric point, where it could be further enhanced by the decrease of the zeta potential, which produces a lessening of the intermolecular repulsion.

The increment of positive protein charge by formation of zinc complexes may thus be considered the main reason for the difference between R_f values in zinc and sodium acetate. Adsorption at the same *p*H's was seen to be signifi-

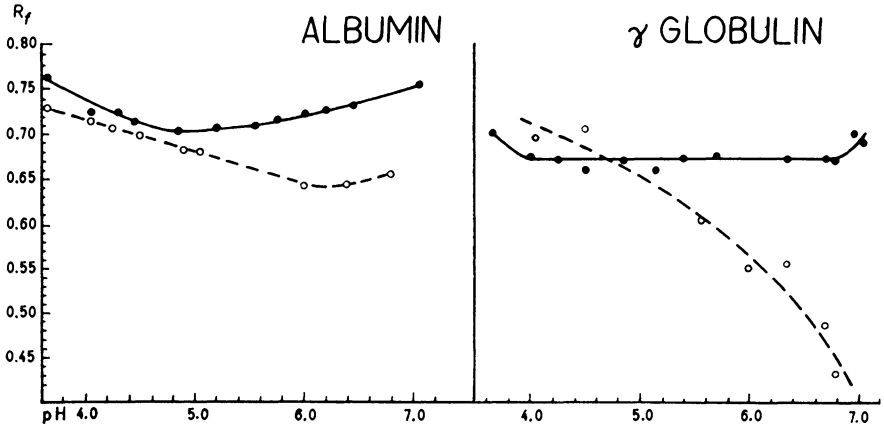

Fig. 1. R_f values of human serum proteins in sodium (● ——●) and zinc (○ - - -○) acetate as function of the *p*H.