in the upper *Lingula*-bearing shale and remain so well into the overlying silty shale. The inferred and SPM paleosalinities in the silty shale and siltstone are in agreement, with the exception of an anomalous set of SPM values in the lower part of the siltstone. These anomalous values may be due to an influx of detrital calcium phosphate, as suggested by Müller (*8*); otherwise they are unexplainable.

Because of the anomalous SPM paleosalinities in the lower *Lingula*-bearing shale, a limiting assumption must be met before Nelson's method can be used to estimate paleosalinities. This assumption is that all phosphate in the sample is of inorganic origin. Organic calcium phosphate concentrators in modern and ancient environments include the conodonts, phosphatic brachiopods, and vertebrate teeth, bones, dermal plates, and scales. The presence of any of these concentrators in any quantity in brackish and freshwater environments will give rise to erroneously high salinity values. Because many detrital rocks possess extremely variable amounts of calcium phosphate attributable to the above concentrators, this places a severe limitation on the applicability of Nelson's method for the determination of paleosalinity.

Müller (*8*) also pointed out two limitations associated with Nelson's method. The first was that calcium phosphate might be of detrital origin and, hence, a contaminant. Undoubtedly, the presence of detrital apatite or other phosphate minerals could give rise to erroneous estimates of salinities based on sediments deposited in freshwater and brackish environments; however, calcium phosphate of detrital origin is not found in all detrital rocks. Müller's second limitation was that, theoretically, iron phosphate under reducing conditions in the presence of H_2S would be transformed into ferrous sulfide. An examination of Fig. 1 reveals that pyritic sulfur and iron phosphate coexist. In fact, the pyritic sulfur content is highest in the freshwater and brackish portions of the section where the iron phosphate content is also highest. If Müller's theoretical argument is valid, it is difficult to understand how such small quantities of iron phosphate could survive in a reducing environment in which pyritic sulfur was forming.

Thus Nelson's method for the determination of paleosalinity is subject to two limiting assumptions. The first assumption is that all phosphate in the rock is of inorganic origin. Because of the widespread spatial and temporal occurrence of organisms capable of concentrating calcium phosphate, it is seldom possible to satisfy this assumption.

The second assumption, that of Müller, is that phosphatic minerals of detrital origin do not represent a significant portion of the phosphate present in the rock. This assumption will also be difficult to satisfy in detrital rocks. In order for SPM paleosalinities to have any meaning, the samples must be subjected to a complete assay for macro- and microfossils. Even then, comminuted phosphatic material may escape detection. These limiting assumptions are so restrictive that Nelson's method should not be used exclusively for the determination of paleosalinity; it should be used only in conjunction with paleontological evidence and other geochemical techniques.

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Serum Cholesterol Reduction by Chromium in Hypercholesterolemic Rats

Abstract. *The addition of chromium (III) to the drinking water of rats in a normal laboratory environment on a hypercholesterolemic diet resulted in lower serum cholesterol concentrations whether the dietary carbohydrate was either sucrose or starch. However, rats fed the sucrose diet with chromium in drinking water had serum cholesterol concentrations similar to those of rats fed the starch diet without chromium in drinking water.*

Chromium influences growth, longevity, glucose metabolism, lipid metabolism, and protein synthesis (*1*). Curran showed that chromium increased the incorporation of labeled acetate into cholesterol (*2*), but Schroeder's experiments, executed under conditions of rigid exclusion of chromium from the environment, suggested that a chromium deficiency resulted in elevated concentrations of serum cholesterol and aortic lipids, and that these conditions could be remedied by the addition of chromium to the diet.

Table 1. Effect of chromium on serum cholesterol. The results are expressed as the mean ± standard error. There were 15 rats per group.

Diet	Serum cholesterol		Mean
	Sucrose (mg/100 ml)	Starch (mg/100 ml)	
Control	270^{+41}_{-34}	209^{+28}_{-13}	237
Chromium	208^{+13}_{-13}	161^{+16}_{-13}	182
Mean	236	183	

After about 12 months of feeding chromium to rats, the concentrations of serum cholesterol were only slightly lowered (*3*), but after 17 months, the reduction in serum cholesterol became significant (*4*). Additional experiments only confirmed a trend toward lower concentrations (*5*). Schroeder's most recent experiments offered evidence that rats fed sucrose diets without chromium showed elevated concentrations

Table 2. Mean weight gain and food consumption. Results are expressed as the mean ± S.E.

Diet	Sucrose		Starch	
	Weight gain (g)	Food intake (g)	Weight gain (g)	Food intake (g)
Control	142 ± 8.5	505 ± 20.5	149 ± 7.2	643 ± 32.4
Chromium	133 ± 11.4	519 ± 29.0	155 ± 9.5	579 ± 24.2

of serum cholesterol, and that adding chromium (III) to this diet lowered cholesterol (*6*). Limited data from humans fed chromium indicated that chromium influenced the lowering of cholesterol concentrations, but the effect was inconsistent (*1*). We investigated the effects of dietary carbohydrates on hypercholesterolemia and a possible relationship of chromium and sucrose on serum cholesterol.

The study of hypercholesterolemia in the rat is facilitated by the addition of cholesterol and cholic acid to the diet. Such diets also containing sucrose or cooked potato starch as the carbohydrate (*7*) were fed to young adult male rats with no effort to control the chromium environment. Both sucrose and starch were fed because there is some evidence that a sucrose diet depletes body chromium stores (*8*) and causes higher concentrations of serum cholesterol (*7*). Chromic acetate was added to the drinking water of the experimental groups to supply 5 ppm of chromium (III) in the water. The same diets were fed to both the control and experimental animals, which were individually housed in raised galvanized cages with wire-mesh bottoms. Food and water were always available. All conditions were identical to those reported (*7*). The total chromium in the sucrose diet was 0.31 ppm and in the starch diet was 0.38 ppm, as determined by atomic absorption spectroscopy after the sample was subjected to dry ashing at 450°C. The cholesterol data were tested for significance by analysis of variance ($P \leq .05$). The standard deviations of the cholesterol values varied directly as the means; therefore, for statistical analysis, the cholesterol values were transformed to logarithms. The values presented were obtained by reconverting the data to their antilogs. The asymmetric distribution of the standard errors around their means results from the reconversion.

The serum cholesterol concentrations after an average of 35 days of feeding of the diets are shown in Table 1. The addition of chromium (5 ppm) to the drinking water significantly lowered the mean serum cholesterol. Chromium supplementation had the same effect when combined with either sucrose or starch. Chromium lowered the mean serum cholesterol in animals eating starch as well as those eating sucrose. Adding chromium to the drinking water of animals fed diets containing sucrose led to concentrations of serum cholesterol similar to the concentrations in the animals fed starch but not receiving chromium. Also the mean serum cholesterol of animals fed the starch diet is significantly lower than the mean of those fed the sucrose diet (*7*).

The mean weight gain and food consumption of each group during the first 28 days of the experiment before the animals were killed are shown in Table 2. The weight gain and food consumption of animals fed the starch-containing diets were slightly higher than those fed sucrose. The increased intake of the diet containing starch only gave a very minimum additional intake of total chromium, and it seems unlikely that this caused the significantly lower serum cholesterol in animals fed starch. These animals drank about 200 ml of water per week, and therefore the experimental groups received 4 to 5 mg of chromium (III) from the water during this 28-day period. The total amount of chromium received from the sucrose diet was 0.16 mg while the starch diet provided 0.24 mg over this same 28-day period.

The results of this experiment confirm and extend Schroeder's finding that chromium can lower serum cholesterol. Since chromium lowers the serum cholesterol of hypercholesterolemic rats housed in a normal laboratory environment whether they are eating starch or sucrose, we conclude that the elevation of cholesterol on sucrose diets cannot be due to chromium depletion alone and that chromium will lower the serum cholesterol concentration with any carbohydrate in the diet.

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Cell Sorting: Automated Separation of Mammalian Cells as a Function of Intracellular Fluorescence

Abstract. *A system for high-speed sorting of fluorescent cells was able to sort mouse spleen cells from Chinese hamster ovarian cells after development of fluorochromasia. Highly fluorescent fractions separated after similar treatment from mouse spleen cells immunized to sheep erythrocytes were enriched in antibody-producing cells by factors of 4 to 10.*

Separation of large numbers of functionally different cell types from the complex mixtures found in such organs as spleen, bone marrow, lymph nodes, liver, or kidney would be useful in biological and biochemical investigations. Direct fractionation methods, such as differential or isopycnic centrifugation, column fractionation, electrophoresis, and so forth, allow separation of large numbers of cells but produce only limited resolution of functionally different types. We are exploring a directed cell-sorting method where various parameters or criteria for separation can be used with a common sorting mechanism based on electrostatic deflection of charged droplets containing the cells. Here we report the use of intracellular fluorescence developed after exposure to the fluorogenic esterase substrate, fluorescein diacetate (FDA), as the parameter for directed sorting of cultured Chinese hamster ovarian (CHO) tumor cells from mouse spleen cells, and for concentration of antibody-producing hemolytic plaque-forming cells from spleens of mice sensitized to sheep red blood cells.

Live cells incubated with FDA accumulate fluorescein intracellularly since FDA can enter the cells and be hydrolyzed by intracellular esterases, but the fluorescein thus produced cannot readily leave through intact cell membranes. This phenomenon has been called "fluorochromasia" (*1*).

In these experiments a suspension of cells, after FDA treatment, passes through a small glass nozzle, forming a liquid stream. This stream is dark-field illuminated by an exciting beam of blue light from a mercury arc source, and the yellow-green fluores-