the importance of variation among kidney cell preparations.

The calculated plaque titer of the "RNA" plus $CaHPO_4 \cdot 2H_2O$ on calcium-depleted cells is independent of "RNA" concentration inoculated if the subsequent dilutions are made into diluent without facilitator but dependent on inoculum concentration when the diluent contains facilitator (Fig. 1). With 0.25 percent $CaHPO_4 \cdot 2H_2O$ in subsequent diluents, as the inoculum "RNA" concentration is decreased, the calculated plaque titer increases and then asymptotes at a value about twenty-five times the titer obtained when the inoculum "RNA" concentration is at the high value of 0.25. Without facilitator in subsequent diluents, the calculated titer, though independent of "RNA" concentration inoculated, is, strictly speaking, *not an "RNA" titer but is a titer of* $CaHPO_4 \cdot 2H_2O$, since the number of plaques produced is limited by the concentration of this phosphate.

Poliovirus "RNA" facilitated by 0.25 percent $CaHPO_4 \cdot 2H_2O$ and inoculated at relatively low "RNA" concentrations onto calcium-depleted kidney cells titers about 10^{-3} of the titer of the intact virus from which it was prepared. It may be possible to raise this relative titer of the "RNA" still further by (i) more severe depletion of the kidney cell of its calcium or (ii) the use of some facilitator other than $CaHPO_4 \cdot 2H_2O$, perhaps at a higher concentration, or both (i) and (ii).

Poliovirus "RNA" very probably lacks, wholly or partially, the natural cell-entry mechanism possessed by intact poliovirus. A sensitive system for the biological assay of "RNA" then probably must provide a substitute mechanism, which could derive from increasing either the receptivity of the cell or the efficaciousness of the "RNA," or both. We have increased both for providing what is probably a substitute cell-entry mechanism for the poliovirus "RNA" (*7*).

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Table 1. The effects of varying four facets of the environment on the number of plaques produced by poliovirus "ribonucleic acid" ("RNA") on monkey kidney tissue cultures. Horizontal spaces separate experiments within which "RNA" concentration in inoculum was constant. Tonicity of media are expressed relative to that of 0.154*M* NaCl (*I*).

Tonicity of medium for "RNA"	Poorly soluble compound added to "RNA"†	Tonicity of medium for cells‡	Cation of which cells are depleted§	No. of plaques per plate*	
				Individual plates	Arithmetic mean
0.90 *I*	None	*I*	None	1;1	1.0
0.90 *I*	None	4.4 *I*	*h*	0;3	1.5
4.0 *I*‖	None	*I*	None	2;2;5	3.0
4.0 *I*‖	None	4.4 *I*	*h*	0;0;0	<0.3
0.75 *I*	None	*I*	None	3;0;1	1.3
0.75 *I*	*P*	*I*	None	11;8;10	9.7
0.75 *I*	None	*I*	None(TT)	0;0;0;1;1	0.4
0.75 *I*	None	*I*	Ca^{++}	0;2;0;0	0.5
0.75 *I*	None	*I*	Mg^{++}	0;1;0;0	0.2
0.75 *I*	None	*I*	Ca^{++} and Mg^{++}	0;0;0;0	<0.2
0.75 *I*	*P*	*I*	None(TT)	1;3;1;1	1.5
0.75 *I*	*P*	*I*	Ca^{++}	30;27;21;34	28.0
0.75 *I*	*P*	*I*	Mg^{++}	11;6;2;5	6.0
0.75 *I*	*P*	*I*	Ca^{++} and Mg^{++}	27;23;29;17	24.0
0.25 *I*	*P*	*I*	Ca^{++}	37;23;16;29;22;13	23.3
0.35 *I*	*P*	*I*	Ca^{++}	44;24;28;42;30	33.6
0.75 *I*	*P*	*I*	Ca^{++}	26;21;33;22;48;9	26.5
2.8 *I*	*P*	*I*	Ca^{++}	4;6;6;12;5;5	6.3
6.7 *I*	*P*	*I*	Ca^{++}	5;1;3;4;5;3	3.5
0.75 *I*	*P*	0.17 *I*	Ca^{++}	3;1;3;4;4	3.0
0.75 *I*	*P*	0.50 *I*	Ca^{++}	22;24;23;30	24.8
0.75 *I*	*P*	*I*	Ca^{++}	23;22;36;22;26	25.8
0.75 *I*	*P*	2.0 *I*	Ca^{++}	9;13;9	10.3
0.75 *I*	*P*	5.0 *I*	Ca^{++}	2;1;6;0;1	2.0
0.75 *I*	None	*I*	Ca^{++}	1;1;0	0.7
0.75 *I*	*R*, 0.012%	*I*	Ca^{++}	5;6;3	4.7
0.75 *I*	*R*, 0.50%	*I*	Ca^{++}	55;52;36	47.7
0.75 *I*	*R*, 1.0%	*I*	Ca^{++}	160;84;100	114.7
0.75 *I*	*R*, 1.5%	*I*	Ca^{++}	63;117;84	88.0

* 0.30 ml inoculated per plate.
† "*P*" means 0.25 percent $CaHPO_4 \cdot 2H_2O$ in the inoculum. "*R*" means Cr_2O_3; the percent values are concentrations of Cr_2O_3 in inoculum.
‡ The two values "4.4 *I*" are for the hypertonic medium (HM) described by Mountain and Alexander (*8*).
§ Only the metallic cations present in the phosphate-buffered saline (PBS) used by Dulbecco and Vogt (*9*), namely Na^+, K^+, Ca^{++}, and Mg^{++}, are here considered. Cells were depleted by washing with PBS without either Ca^{++} or Mg^{++} or without both, incubating ½ hour at 37°C, and rewashing with the kind of medium used for the first washing. Many of the cells depleted in this way of both these divalent cations or of Ca^{++} alone are rounded. The designation "None(TT)" indicates "time-temperature" control cultures whose cells were treated the same as the depleted cells except that PBS was used. The designation "None" indicates cultures which were washed with PBS. Designation *h* indicates cultures which were washed with PBS, incubated in HM for ½ hour at 37°C, and inoculated after removal of HM but without rewashing; these cultures may have been partially depleted of one or more of the cations K^+, Ca^{++}, and Mg^{++}.
‖ HM was the main component.

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Cytochrome c Reductase of Tri- and Diphosphopyridine Nucleotides in Rat Lens

Abstract. The ocular lens of the 28- to 32-day-old rat contains an active hexose monophosphate shunt pathway for the combustion of glucose. Triphosphopyridine nucleotide (TPNH) cytochrome *c* reductase is present in this organ and is approximately one-third more active than diphosphopyridine nucleotide (DPNH) cytochrome *c* reductase. Since there is no transhydrogenase activity in these lenses, and since DPNH lactic dehydrogenase is 15 times as active as TPNH lactic dehydrogenase, the presence of an active TPNH cytochrome *c* reductase may provide this organ with the means of reoxidizing the relatively large amounts of TPNH formed by the direct oxidative pathway of glucose metabolism. Although TPNH oxidation in other tissues has not as yet been shown to yield adenosine triphosphate (ATP) directly, it is possible that such a mechanism may be operative in the rat lens.

Previous experiments have indicated that the hexose monophosphate shunt may be an important pathway of carbohydrate metabolism in the lens of the young rat (*1*). Recent studies to determine the levels of oxidized and reduced di- and triphosphopyridine nucleotides (DPN, TPN, DPNH, and TPNH) have shown that the TPNH:TPN ratio in the

lens derived from the young rat (aged 28 to 32 days) is 5.69 as compared with a DPNH:DPN ratio of 1.52. (*2*) These results provide further evidence of an active shunt pathway in this organ.

There must be some mechanism within the lens to oxidize the relatively large amounts of TPNH formed during the first two steps of glucose oxidation via the shunt. Employing the method of Kaplan *et al.* (*3*) and Stein *et al.* (*4*) in which DPN analogues are used, I was unable to demonstrate by repeated assays any transhydrogenase activity within the rat lens. Although a relatively inactive TPNH-linked lactic dehydrogenase is present in this organ, its activity is only approximately 1/15 the activity of DPN lactic dehydrogenase (*5*) at *p*H 7.4, and it is difficult to see how such a system could account for an efficient and rapid reoxidation of TPNH.

TPNH and DPNH cytochrome *c* dehydrogenase were then looked for in the lens. Pirie *et al.* (*6*) have reported that TPNH cytochrome *c* dehydrogenase activity is present in the rat lens. Employing their assay system, I discovered that cytochrome *c* could be reduced very rapidly if only an aliquot of a lens homogenate—and no TPNH or DPNH—was added to the cuvette. Four lenses derived from 28- to 32-day-old Holtzman strain white male rats were therefore homogenized in 5 to 10 times their weight in deionized water, dialized overnight, and centrifuged; and the supernatant solution was retained for the assay procedure. Since an aliquot of this supernatant solution was also capable of rapidly reducing cytochrome *c* (without any added DPNH or TPNH), the solutions were assayed for any ascorbate that might still be present in a bound form. The Roe procedure based on the reduction of the dye 2,6 dichlorophenolindophenol (*7*) was employed as a simple assay for ascorbate, and the results showed that no ascorbate was present in these dialized solutions. There were also no sulfhydryl groups present (for example, reduced glutathione or cysteine) in sufficient quantity to account for the degree of cytochrome *c* reduction. Heating the solution for 2 minutes at 100°C completely destroyed its activity. Studies are now in progress to further characterize and determine this compound.

In order to assay for TPNH and DPNH cytochrome *c* activity, it was thus necessary to add a large excess of cytochrome *c* (type III Sigma) to the test system and allow the initial reduction of cytochrome *c* to go to completion. Approximately 30 to 45 seconds were required for this to occur. Either TPNH or DPNH was then added to the cuvette, and further reduction of cytochrome *c* was determined for a 5-minute interval. The results of such assays on four samples obtained from 16 rat lenses for TPNH and four samples (16 lenses) for DPNH cytochrome *c* reductase activity are shown in Table 1. Both TPNH and DPNH cytochrome *c* reductases are present within the rat lens, and the former is apparently about 30 percent more active (*8*).

Table 1. Results of assays for TPNH and DPNH cytochrome *c* reductase activity. The enzyme activity is expressed as the change in optical density at 550 mμ of 0.001 per minute at 24°C per lens.

Lenses (No.)	Av. wet weight per lens (mg)	Cytochrome *c* reductase activity	
		TPNH	DPNH
4	19.10	14.40	6.91
4	19.20	17.75	14.40
4	19.02	19.72	14.79
4	19.60	19.72	14.709
Mean activity		17.90	12.72

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Estimation of Total Body Fat from Potassium-40 Content

Abstract. On the assumption that the potassium content of the lean body mass is constant, it should be possible to estimate fat content in living man from a measurement of potassium-40 activity in the whole-body scintillation counter. A series of such measurements on children and young adults shows good correlations with skin-fold thickness and weight/height ratio as indices of fatness.

Current interest in obesity has resulted in attempts to assess the actual fat content of the body in living human subjects. Methods now in use include calculations based on measurement of total body water and body density (*1*) as well as inferences drawn from measurements of skin-fold thickness. The assumptions involved in the first two methods, and their inherent limitations, have recently been discussed (*2*).

The purpose of our report is to suggest a new approach to this problem, namely the use of whole-body potassium content as an index of lean body mass. This approach is based on results of chemical analyses of adult human subjects; only four such analyses have been done, and these revealed values of 66.5, 66.6, 72.8, and 66.8 meq/kg of lean body weight (*3*) (this term is taken to mean body weight minus chemically determined neutral fat). Other workers have emphasized the relative constancy of whole-body potassium content in such species as the cat, rabbit, rat, and pig when values are expressed on a fat-free basis (*4*), though the absolute values are somewhat higher than those for man. There is a high correlation between K^{40} content and lean tissue mass in hams (*5*). Woodward *et al.* (*6*) found a good correspondence between K^{40} content and lean body mass as determined by tritium dilution in man. Meneely *et al.* (*7*) were able to correlate K^{40} activity with basal heat production. The assumption of a constant potassium content for the lean body mass would thus appear to be a reasonable one.

Fifty subjects were accordingly assayed for K^{40} in a whole-body scintillation counter, and the estimates of fat content so derived were correlated with skin-fold thickness and weight/height ratio.

The subjects comprised 42 males, aged 11 to 44 years, and 8 females, aged 7 to 23 years. Body weights ranged from 22 to 105 kg. All were judged to be in good physical health. An attempt was made to include both obese and thin subjects so the results cannot be taken as representative of the normal population. Subjects were dressed only in a light cotton gown and paper slippers at the time of measurement. Skin-fold thickness was determined at six locations using special calipers (*8*): mid-biceps, mid-triceps, abdomen below and 1 cm to left of umbilicus, subcostal at nipple line, iliac crest, and subscapular.

Potassium-40 measurements were made in a whole body scintillation counter, according to the technique described in detail by Miller (*9*). The subject reclines in a specially constructed metal chair and is viewed by an 8-inch diameter, 4-inch thick sodium iodide crystal. The gamma rays emitted by the subject, which penetrate the crystal, produce scintillations which are seen by four 3-inch photo multiplier tubes. The signal output from the pho-

Cytochrome c Reductase of Tri- and Diphosphopyridine Nucleotides in Rat Lens

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