(240 days), if they have three feeding cycles a day, and if calcareous fragments in the gut constitute 3 percent of the body weight, then the fishes must redeposit 1080 kg (roughly 1 ton) of calcareous material per hectare per year (3 percent of 55 kg $\times$ 3 $\times$ 240).

Analogous calculations have indicated that one or two daily fillings of the digestive tracts yield about 700 kg of material per hectare per year from surgeonfishes and 600 kg from parrot fishes. The larger filefishes were disregarded because they were present in relatively small numbers on the study reef. Where they occur in greater numbers they should be included in such an estimate because they feed almost exclusively on the tips of branching corals (*2*).

Adding the weight of calcareous material, purposely or accidentally ingested, which passes through the gut of small, medium-sized, and larger browsing reef fishes, one arrives at a total weight of at least 2300 kg/hectare yr.

Possible sources of error in this study lie in the following factors.

1) The estimate of numbers of fishes present. This source of error was discussed in a previous publication (*4*), where it was suggested that the estimate was on the low rather than on the high side.

2) The indicated effect of temperature on feeding. While such effects may be considerable in Bermuda, where corals and certain reef fishes live at the northern margin of their range, they should not be noticeable in truly tropical circumequatorial reef regions.

3) The omission of certain additional groups of fishes which only rarely ingest sand and coral or calcareous algal fragments (for example, jacks, Carangidae). For Bermuda this omission would tend to counteract an error on the high side resulting from reductions in feeding during the winter months, but for more tropical regions the omission would make my estimate of the amount of redeposited material too low.

4) The occurrence of periods of nonfeeding—for instance, during spawning. I do not believe this to be a large consideration because (i) parrot fishes were observed to feed in the spawning season, on one occasion even between successive pairing acts, and (ii) the nutritive content of attached algae forces fish which feed largely or partly on such materials to take in substantial quantities of food to sustain themselves, to say nothing of growing (*5*, *6*).

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Facilitation of Infection of Monkey Cells with Poliovirus "Ribonucleic Acid"

Abstract. The plaque titer of poliovirus "ribonucleic acid" on monkey kidney cells cultured in vitro is greatly increased by depleting these cells of calcium and treating the "ribonucleic acid" inoculum with a suspension of any one of several poorly water-soluble substances before inoculation. These undissolved substances apparently facilitate infection by serving as solid vehicles for the "ribonucleic acid."

Intact ribonuclease-stable poliovirus is changed by phenol into an infective unit destructible with ribonuclease; this ribonuclease-labile poliovirus unit is called poliovirus "ribonucleic acid (RNA)" (*1*). When standard plaque assay techniques are used poliovirus "RNA" manifests a plaque titer on monkey kidney tissue cultures of only about $10^{-6.5}$ of the titer of the intact virus from which it was prepared (*2*). In this report we show how the titer of poliovirus "RNA" on such kidney cells can be greatly increased (*3*).

Poliovirus "RNA" was obtained by one treatment of intact wild-type virus of the Brunhilde strain (antigenic type 1) at 0° with 7 percent water-saturated phenol (*2*); this method is a modification of method of Gierer and Schramm (*4*). Kidney cells were grown and maintained and poliovirus stocks were obtained as described previously (*2*).

The combination of two specific procedures results in a large increase in the number of plaques produced by poliovirus "RNA." These two procedures are (i) addition to the "RNA" of any one of several compounds of low solubility in water, and (ii) depletion of the kidney cells of calcium (Table 1). Poorly soluble substances which facilitate infection of calcium-depleted cells with "RNA" include, besides $CaHPO_4 \cdot 2H_2O$ (*5*) and Cr_2O_3 (Table 1), Al_2O_3, $CaCO_3$, $CaSO_4$, Co_2O_3, Fe_2O_3, Fuller's earth, $MgCO_3$, MgF_2, $MgHPO_4 \cdot 3H_2O$, $Mg_3(PO_4)_2 \cdot 5H_2O$, $Mg_3Si_4O_{11} \cdot H_2O$, NiO, and ZnS. Two of these facilitators were tested for their capacity to adsorb poliovirus "RNA": at a facilitator concentration of 0.25 percent, $CaHPO_4 \cdot 2H_2O$ adsorbed 56 percent and $Mg_3(PO_4)_2 \cdot 5H_2O$ adsorbed 67 percent of the "RNA."

With $CaHPO_4 \cdot 2H_2O$ as facilitator, and calcium-depleted cells, the number of plaques formed is dependent on the tonicity both of the medium used for the "RNA" and of the medium used for the cells (Table 1) (see *6*). When the medium for the cells is isotonic fewer plaques are formed when the medium for the "RNA" is hypertonic than when it is isotonic or hypotonic. When the medium for the "RNA" is slightly hypotonic, peak plaque production is obtained when the medium for the cells is slightly hypotonic.

With $CaHPO_4 \cdot 2H_2O$ as facilitator, and calcium-depleted cells, the number of plaques produced by the "RNA" was dependent on the duration of the post-inoculation incubation at 37°C before layering with the nutritional agar maintenance medium. The results of three experiments suggest that the largest number of plaques is obtained when this duration approximates 1 hour. The moderately large variation among these experiments, however, suggests

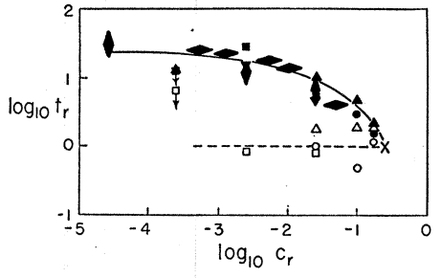

Fig. 1. The relationship of calculated plaque titer of poliovirus "RNA" with "RNA" concentration inoculated. Symbol "c_r" means relative concentration of "RNA" inoculated, based on assigning unity concentration value to "RNA" concentration in undiluted "RNA" preparation. Symbol "t_r" means calculated relative titer of "RNA," based on assigning unity titer value to titer obtained when $c_r = 0.25$. For $c_r = 0.25$, denoted by *X*, and for all solid symbols inoculum contained 0.25 percent $CaHPO_4 \cdot 2H_2O$; for all open symbols inoculum contained <0.25 percent $CaHPO_4 \cdot 2H_2O$ since these inocula were obtained by dilution of the mixture of "RNA" and $CaHPO_4 \cdot 2H_2O$ into diluent(s) without $CaHPO_4 \cdot 2H_2O$. All symbols of same shape from same experiment, of different shape from different experiments. Arrows denote maximal points; that is, no plaques were found at inoculum "RNA" concentration indicated.

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