designated AHT, may be related etiologically to human caries. Thus far no attempt has been made to reproduce caries in the human mouth using this agent.

Eleven caries-free human mouths have been cultured and in no instances were streptococci similar to the AHT, BHT, or CHT observed.

Summary. Streptococci were isolated from human dental carious lesions which reacted with fluorescein-tagged antisera against rat or hamster "cariogenic" streptococci. The human strains fell into 3 different categories, in fluorescence, morphology, microprecipitin and gel diffusion studies. One category, similar to the hamster strain, produced caries when used to infect hamsters. This occurred in 55 animals of 55 tested, and in no controls. The other 2 categories of human isolates failed to produce hamster caries; one of these is being studied for possible cariogenicity in germfree rats.

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Growth Stimulating Effects of Acid Mucopolysaccharides.* (29965)

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A preliminary report from this laboratory (1) has shown that certain acid mucopolysaccharides (AMPS) and calf aorta extracts (containing a mixture of AMPS) have growth stimulating effects and lipid reducing activity by biochemical determinations in lipid saturated chick aorta cells and HeLa cells. However, the reduction of cellular lipid was accompanied by cell growth as stimulated by AMPS or crude calf aorta extracts rich in AMPS(1). Other investigators employing histochemical techniques(2,3) have also described the acid mucopolysaccharide heparin as having a lipid-clearing effect upon lipid-loaded aortic cells.

This communication describes the effects of certain AMPS, calf aorta extracts, and phospholipid on HeLa cell growth. Studies of large volumes of HeLa cells have made it possible to relate cytocrits, cell counts, cell volumes, and dry weights on a quantitative basis.

Materials and methods. The HeLa cells were diluted 1:1000 v/v in Eagle's Minimum Essential Medium with 5% calf serum, planted 50 ml in 1-liter and 100 ml in 2-liter bottles, and grown for a total of 13 days. After initial growth of the cell cultures for 7 days, human lipemic serum (HLS) was added in 10% concentrations and incubated for an additional 3-day period. The medium was then replaced with fresh Eagle's Minimum Essential Medium containing the substances to be tested, and this was renewed daily for 3 days. The following test substances were employed(1): calf aorta extracts (Extract #1

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TABLE I. Effect of Acid Mucopolysaccharides and Other Test Substances on HeLa Cell Growth as Measured by Cytocrit Determinations. All test groups were treated with human lipemic serum.

Test substance	Amt in mg %	No. determinations	Growth in %* Mean	Standard error	P value†
Normal control	—	19	93.5	1.6	<.001
Extract #1	8	11	108.8	3.8	<.05
" #4	33	10	111.3	2.4	<.01
Chondroitin sulfate A	1	6	121.7	5.0	<.01
" " C	1	6	118.3	5.9	<.05
Phospholipid	1	6	109.8	3.5	<.05

* Numbers express a comparison of the test group with HLS control from the corresponding group. Total of 21 experiments of HLS control; mean of each group of HLS controls taken as 100%.

† P value expresses comparison of the difference of mean values of test groups and corresponding HLS controls.

and Extract #4, Riker Laboratories†); chondroitin sulfates A or C (CS-A, Riker Laboratories, and CS-C, Dr. K. Meyer); keratosulfate (Dr. K. Meyer); phospholipid (PL, Phillips Roxanne Co.).

Following exposure to the test substances, the cells were washed, trypsinized(4), centrifuged, and adjusted to 5 ml with calcium and magnesium free salt solution. A small amount of this cell suspension was drawn into a 150 mm capillary tube and centrifuged for 15 minutes at 2,000 rpm for cytocrit determinations; hemocytometer cell counts were made from the same cell suspension. Remaining cell harvests were measured for total dry weight. Each experiment contained normal and lipemic serum controls, as well as one or more of the above test substances. Since variations between experiments were of fairly large magnitude, comparisons were made after data were expressed as percentile value of the HLS control.

Table I shows the result of the effects of the test substances on cell growth as measured by cytocrit determinations. HLS was a growth stimulating agent since in almost all experiments cell volume was greater than that of the normal controls. The increase was significant at the 0.001 level. HLS in combination with CS-A, CS-C, calf aorta extracts, or PL, stimulated greater total cell volume than HLS alone. Significant differences were obtained when the HLS average was compared with either HLS plus Extract #4 or CS-A ($P < 0.01$). HLS plus Extract #1, CS-C, or PL also resulted in increased growth when compared with HLS control. In this instance growth is measured by cell volume increase. However, the P value of < 0.05 does not allow one to be as firm in assuming that the differences observed are as meaningful as the P value of < 0.01.

† These represent 2 different saline extracts of acetone dried calf aorta.

Table II shows the effects of the test substances on growth as determined by total dry weights and cell counts. HLS increased total dry weight 9.2% over the normal control ($P<0.05$). CS-A and PL showed the most significant changes when growth was measured by total dry weight. In contrast, Extracts #1 and #4, and HLS alone, showed the most significant changes in growth measured by cell count. In the final column the relative cell volume data indicate that subtle changes, apparent with the cytocrit, are occurring in cell size as well as in cell number. The influence of some of our test substances appears to be primarily related to increase in cell size, wherein CS-A is most pronounced. In contrast, Extract #4 increased the number of small cells. In either case the effect is cell growth by increase in cell number or cell size.

CS-A or CS-C produce a large increase in cytocrit, but relatively small increase in cell numbers; the individual cell volume appears to be increased. On the contrary, PL showed the smallest cell volume. The increase of cell volume with the chondroitin sulfates may be correlated with an increase of total RNA and the RNA/DNA ratio (findings to be reported later).

TABLE II. Effect of Acid Mucopolysaccharides and Other Test Substances on HeLa Cell Growth as Measured by Total Dry Weight, Cell Count, Cell Volume. All test groups were treated with human lipemic serum.

Test substance	No. determinations	Total dry wt* Mean	Total dry wt* Standard error	Total dry wt* P value†	Cell No.* Mean	Cell No.* Standard error	Cell No.* P value†	Relative cell vol, cytocrit in %/ cell No. in %
Normal control	14	90.8	3.3	<.05	86.7	.2	<.001	1.08
Extract #1	7	112.1	4.5	<.05	115.3	4.5	<.01	.94
" #4	5	110.9	3.3	<.05	123.1	4.1	<.001	.90
Chondroitin sulfate A	6	112.3	2.4	<.001	109.2	2.8	<.05	1.11
Chondroitin sulfate C	5	103.2	1.9	>.05	112.6	2.7	<.05	1.05
Phospholipid	4	113.4	1.1	<.001	128.3	5.7	<.05	.86

* Numbers express a comparison of the test group with the corresponding HLS control group. 16 experiments of HLS in total; mean of each group of HLS controls taken as 100% or as a relative cell volume of 1.00.

† P value expresses comparison of the difference of mean values of test groups and corresponding HLS controls.

It appears that certain AMPS have appreciable growth stimulation in the HeLa cell systems. A comparable effect has previously been demonstrated in chicken aorta cells treated with extracts of calf aorta(1). Although decreases in relative amounts of lipid may not always accompany cell growth, the stimulating effect of AMPS may play a role in the lipid metabolic cycle. The degrees of stimulation of cellular activity which the various test substances demonstrate may influence different stages of intracellular metabolic activity and this is being investigated at present.

Summary. The growth stimulating effect of acid mucopolysaccharides on lipid saturated HeLa cell cultures has been examined by measurement of cytocrits, total dry weights, and cell counts; results demonstrate that certain AMPS have a significant growth stimulating effect.

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Dynamics of Amino Acid Transport in the Intact Intestine.*† (29966)

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This laboratory has reported a 2-way exchange of L-tyrosine across the intestinal mucosa of the intact rat(1). We have also

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† Abbreviations and special terms used in this paper are as follows: Glycine (Gly), Alanine (Ala), Serine (Ser), Proline (Pro), Valine (Val), Threonine (Thr), Isoleucine (Ileu), Leucine (Leu), Methionine (Met), Phenylalanine (Phe), Tyrosine (Tyr), Aspartic Acid, (Asp), Glutamic Acid (Glu), Lysine (Lys), Histidine (His), Arginine (Arg). The term *efflux* refers to an outward movement of the amino acid into the lumen and perfusate; *perfusate* being the solution circulated through the cannulated lumen of an intestinal segment; *preperfusate*, the above solution before perfusion.