TABLE I. Correlations between *in Vivo* and *in Vitro* Assays for Streptovitacin A.

Streptovitacin A preparation	Relative activities		
	Tissue culture	WAC*	*S. pastorianus*
1 (CML-70)	7	10	5
2 (TEE-98)	55	70	40
3 (crystalline)	100	100	100

* Walker-256, in rats (Evans, J. S., unpublished).

removed, it was a suitable assay for isolation and purification of streptovitacin A from fermentation beers. Total assay time was 5 days. This study emphasizes the rapidity, utility, sensitivity, and reproducibility of tissue culture assays for antitumor agents present in fermentation beers, provided a correlation exists between *in vivo* and *in vitro* activities.

Summary. A tissue culture assay employing Eagle's KB epidermoid carcinoma has been described which measured streptovitacin A concentrations between 0.02 and 0.25 μg/ml. Tissue culture assay measured streptovitacin A potency in crude beers and extraction preparations, in addition to crystalline material. Cycloheximide was also inhibitory to KB cells with an ID_{50} of 0.1 μg/ml. Standard error per ID_{50} determination was 15%, and per relative potency determination, 8%.

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Serum Lipid, Lipoprotein and Vascular Tissue Studies in Cholesterol-Fed Horse.* (24769)

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Shorland(1) noted that depot fats of various pasture-fed animals reflect the composition of dietary fats to different degrees. Body fats in horses and wild rabbits included 16 and 40% of linolenic acid respectively while fats from oxen, sheep and deer (ruminants) contained less than 1% (all animals having been at pasture in rye-grass, glycerides of which contained 63% of linolenic acid). This similarity of effect of dietary linolenate on fat depots of horses and rabbits prompted us to determine if cholesterol feeding would cause hypercholesterolemia and vascular lesions in the horse since this phenomenon is very striking in rabbits.

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Methods. A 1275 lb gelding approximately 12 years old and in apparent good health (cataract in one eye) was fed prairie hay and ½ U.S. peck of caramelized oats ("Carmol-oats") daily. U.S.P. cholesterol, 225 g daily, (estimated to constitute 1.7-2% of diet) was added to oats during 3 month period. The horse received no regular exercise. Blood was obtained by jugular puncture. Serum lipids and lipoproteins were measured before, during, and after cholesterol supplementation period. Total and free cholesterol were determined by the method of Sperry and Webb(2) and lipid phosphorus by modification (Hawk *et al.*,(3)) of the method of Youngburg and Youngburg. Lipoproteins were determined refractometrically at solvent density 1.21 g/ml after preparative centrifugation for 22 hours at 40,000

TABLE I. Serum Lipid and Lipoprotein Values.

Date	Total cholesterol, mg %	% ester cholesterol	Lipid P, mg %	Ratio: Cholesterol/lipid P	Lipoproteins, mg % $-S_{1.21}>70$	$-S_{1.21}20$-70	$-S_{1.21}0$-20
7/ 5/57	104.5	79.3	6.6	15.8	0	96	256
18	97.2	82.6	5.8	16.8	19	83	217
27	93.6	80.4	6.3	14.9	45	108	223
9/ 6	91.7	82.1	5.9	15.6	26	77	179
Mean ± S.E.	96.8	81.1	6.2	15.8	23	91	219
	± 2.8	±.8	±.2	±.4	± 9	± 7	± 16
			Cholesterol feeding commenced 9/6/57				
9-12/57	98.0	80.5	6.1	16.1			
25	98.8	77.9	6.4	15.4	50	79	203
10/10	82.5	78.3	5.4	15.3	15	67	199
23	80.1	79.5	5.2	15.4	10	90	177
11/12	100.3	78.7	6.2	16.2			
Mean ± S.E.	91.9	79.0	5.9	15.7	25	79	193
	± 4.4	±.5	±.2	±.2	± 13	± 7	± 8
p_1*	n.s.	.02	n.s.	n.s.	n.s.	n.s.	n.s.
			Cholesterol feeding ceased 12/2/57				
12/ 3/57	112.4	81.0	6.8	16.5	26	75	270
17	123.1	85.2	7.3	16.9	15	101	246
31	118.2	77.7	6.6	17.9	28	97	263
1/14/58	125.7	80.0	7.0	17.9	33	141	275
2/ 5	125.8	79.7	7.0	18.0	35	113	246
18	124.7	77.8	7.3	17.1			
26	124.2	81.2	7.1	17.5			
Mean ± S.E.	122.0	80.4	7.0	17.4	27	105	260
	± 1.9	± 1.0	±.1	±.2	± 3	± 11	± 6
p_2*	.001	n.s.	.02	.01	n.s.	n.s.	.01
p_3*	"	"	.01	.001	"	"	.001
2/27/58	Horse sacrificed						

* p_1, significance of difference between cholesterol feeding and control periods (n.s. = not significant).
p_2, Significance of difference between post cholesterol feeding and control periods.
p_3, *Idem* cholesterol feeding periods.

rpm in Spinco #40 rotor according to methods previously reported(4). Values obtained indicate relative amounts of different classes of serum lipoproteins. Fecal analyses were usually carried out on 24 hour specimens. Triplicate 50 g samples of comminuted specimens were acidified with concentrated HCl, extracted twice with boiling ethanol and 9 times with boiling 1:1 (v/v) ethanol-ether. Solvent was then removed under reduced pressure and the lipids reextracted with petroleum ether (30-60°C boiling range). The extract was washed 3 times with distilled water, dried over anhydrous sodium sulfate, filtered and made to standard volume. The non-fat, extracted, fecal residue was dried in forced-draft oven at 85°C for 7 to 12 hours, cooled and weighed. The extracts were analyzed for total extractables gravimetrically following removal of solvent, and for cholesterol by Sperry and Webb(2) procedure.

Results. Table I lists serum lipid and lipoprotein values. During dietary cholesterol supplementation there were no significant changes in serum lipids and lipoproteins. Following removal of cholesterol from the diet serum cholesterol, lipid P, C/P ratio and $-S_{1.21}$ 0-20 "alpha" lipoproteins underwent small but statistically significant increases. Dates in Table indicate different climatic conditions to which the horse was subjected during experiment.

Fecal analyses data (Table II) indicate 76% net intestinal absorption of cholesterol during cholesterol supplementation period. (Analysis of fecal lipid extracts for cholesterol by the Sperry and Webb procedure agreed very well with values obtained by applying

TABLE II. Intestinal Absorption of Dietary Cholesterol.

Cholesterol added to daily diet	Date	Wet wt, 24 hr feces specimen, g	% extractables, wet wt basis	% non-fat dry material	Cholesterol per g wet wt feces, mg	Cholesterol excreted per 24 hr, g*	Digestive coefficient of cholesterol, %†
225 g	10/18/57		1.96		9.7	52.6	76.6
	11/12	4350	2.19	24.5	10.4	56.4	74.9
None	12/13/57	3100	1.39	23.1	.48	2.6	
	31	8110	1.30	28.5	.39	2.1	
	2/ 6/58	6140	1.33	26.3	.35	1.9	

* Calculated from avg daily feces excretion of 5,425 g.

† Calculated without correcting for endogenous fecal sterol or dietary plant sterol excretion and without taking into account conversion of cholesterol to coprosterol and other sterols.

the Liebermann-Burchard reaction (Sperry and Webb conditions) directly to a sample of total lipids.)

The heart and aorta were removed and examined for gross and microscopic lesions. Oil Red O showed aortic intima unremarkable. Spotty areas of interstitial fat were seen in the media. Alcian blue staining revealed acid mucopolysaccharides in medial ground substance. Elastica appeared normal in aorta and coronary arteries. No abnormality of coronary arteries was noted with Haematoxylin-Eosin. Schultz staining, combined staining (Abu-Haig and Rinehart) and Periodic-Acid-Schiff preparations of coronary arteries and aorta were not remarkable. No myocardial abnormality was noted.

Discussion. The data do not indicate to what factors the increase in serum lipids following cessation of cholesterol-supplementation is attributable. The onset of cold weather, cholesterol feeding, castration, and lack of exercise should be considered. Our studies(5) indicate that an "overswing" phenomenon may occur following removal of long-imposed stimulus to homeostatic mechanisms regulating serum lipid levels. In the horse, dietary cholesterol may have provided the stimulus to the homeostatic mechanism.

Chemical and histological examination of liver and aorta revealed no accumulations of lipids (Liver, wet, : total lipids 43.7 mg/g, cholesterol 1.83 mg/g, lipid P 0.85 mg/g; Aorta, dry, : cholesterol 5.36 mg/g, lipid P 0.573 mg/g. Absence of cholesterol deposition in blood vessels or liver in the presence of good intestinal absorption of cholesterol suggests that the horse metabolizes cholesterol in a manner different from that of the rabbit.

Summary. A diet containing 1.7 - 2% cholesterol did not alter serum lipid or lipoprotein levels when fed to a gelded horse for 3 months. When cholesterol supplementation was discontinued, significant increases in serum total and free cholesterol, lipid phosphorus, C/P ratio and high density $-S_{1.21}$ 0-20 ("alpha") lipoproteins occurred which persisted until sacrifice 3 months later. Fecal lipid analyses indicated net cholesterol absorption in excess of 70%. Liver and aortic lipid analyses and histologic examination of myocardium, coronary arteries and aorta were not remarkable.

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