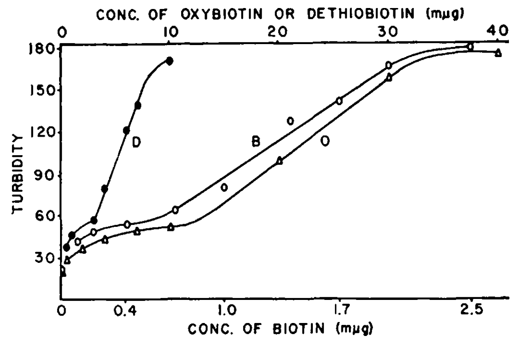

FIG. 3. Response of *E. coli* to biotin, oxybiotin and dethiobiotin. 22 hr incubation at 30°C under stationary conditions. B = D-biotin. O = DL-oxybiotin (o—heterobiotin). D = DL-dethiobiotin.

would appear that CO_2 is an essential substance in the metabolism of *E. coli* and that biotin is concerned with production of metabolic CO_2. The function of biotin in the decarboxylation of oxalacetate and succinate is known(6), and the sparing effect of CO_2 on biotin requirements of other organisms has been reported(5). The fact that the CO_2 effect was greater in the presence of increased amounts of biotin may reflect the utilization of CO_2 in biotin-linked CO_2 fixing systems resulting in formation of substances necessary for optimal growth of this organism.

Microorganisms vary widely in their ability to utilize analogues of biotin. This likely depends both upon specificity and ability to convert the analogue into a usable form. The comparative results obtained with biotin, oxybiotin‡ and dethiobiotin with *E. coli* under stationary conditions of incubation are shown in Fig. 3. It is manifest that both analogues were utilized by *E. coli* although not as effectively as biotin. If one assumes that only the D-isomer is active biologically then the values given for oxybiotin and dethiobiotin should be halved. The reduced activity of both analogues (and especially oxybiotin) suggests either that they are not efficiently converted to biotin by this organism or that they are used metabolically in a form different from that produced from biotin.

‡ We are indebted to Hoffmann-LaRoche, Inc. for a generous supply of this compound.

Summary. The biotin requirements of a biotinless mutant strain of *E. coli* are presented. Data on the influence of degree of aeration and changes in nutrition on the response of this organism to biotin are included.

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Influence of Dietary Sodium Chloride on Incidence of Urinary Calculi in Sheep.* (23359)

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Addition of potassium acid phosphate (K_2HPO_4) to diets fed to sheep has been reported to greatly increase the incidence of urinary calculi(1). Further studies(2) have indicated that both phosphorus and potassium are important in the etiology of urinary calculi in sheep. The largest occurrence of cal-

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TABLE I. Composition of Diets, Lb.

	1	2	3	4	5	6	7	8	9	10	11	12
Wheat straw	44.7	40.5	44.7	40.5	43.8	39.7	43.8	39.7	19.7	18	19.7	18
Sugar	5	4.5	5	4.5	5	4.5	5	4.5				
Ash of beet pulp					.9	.8	.9	.8				
Beet pulp (without molasses)									30	27	30	27
Wheat	15	13.5	15	13.5	15	13.5	15	13.5	15	13.5	15	13.5
Oats	17	15.2	17	15.2	17	15.2	17	15.2	17	15.2	17	15.2
Linseed meal	17.8	16.3	17.8	16.3	17.8	16.3	17.8	16.3	17.8	16.3	17.8	16.3
NaCl	.5	10	.5	10	.5	10	.5	10	.5	10	.5	10
K_2HPO_4			2.4	2.4			2.4	2.4			2.4	2.4
Vit. A and D*												
Calcium, %	.291	.231	.297	.291	.378	.638	.421	.456	.491	.387	.447	.387
Phosphorus, %	.249	.297	.496	.626	.383	.388	.728	.708	.360	.282	.708	.738
Magnesium, %	.153	.133	.143	.128	.259	.178	.120	.184	.240	.215	.115	.208
Potassium, %	.884	.758	1.373	1.474	.751	.671	1.775	1.638	.669	.579	1.654	1.666
Sodium, %	.338	3.350	.325	4.000	.407	2.960	.310	3.920	.362	3.500	.369	2.830
Chloride, %	.843	6.030	.728	6.381	1.062	5.320	.442	5.720	.667	5.939	.843	5.320

* 325 I. U. vit. A and 100 I. U. vit. D/lb of feed.

culi resulted in sheep supplemented with both phosphorus and potassium rather than either one singly. Lindley *et al.*(1) reported that 25% of molasses dried beet pulp in diets fed to sheep increased the incidence of urinary calculi. Later investigations by Elam *et al.* (2) indicated beet pulp rather than molasses in molasses dried beet pulp was responsible for the predisposing effect on urinary calculi formation. However, the factor(s) in beet pulp responsible for this effect is not known. Since surgery is often necessary in the treatment of animals afflicted with the disease, the development of a preventive treatment is desired. That a large intake of sodium chloride causes diuresis in animals is well known. Meyer *et al.*(3) reported that feeding sodium chloride to 9.4% of the diet did not adversely affect the average daily gains or efficiency of feed utilization of fattening sheep and cattle. Further, there is evidence that an interrelationship exists in the body between sodium and potassium(4).

The objectives of this experiment were: (1) to obtain additional information on the factor in beet pulp responsible for its predisposing effect on calculi formation, to observe the effects of adding potassium acid phosphate to the diet, and (2) to study the influence of adding a relatively high level of sodium chloride to the diet on the incidence of urinary calculi.

Procedure. Sixty crossbred wether lambs, averaging 84 lb, were divided into 12 lots of 5 lambs each and group-fed a pelleted diet twice daily. The diets were supplemented with adequate levels of vit. A and D to eliminate the possible influence of a low level of these vitamins on the formation of calculi. Water was available *ad libitum.* Variables in the diets were: potassium acid phosphate (K_2HPO_4), sodium chloride, beet pulp (without molasses), and ash of beet pulp. These variables were included in a factorial design (Table I). Beet pulp ash was fed to determine if the causative factor in beet pulp is inorganic. Beet pulp was supplied at approximately 30% of the diet in 4 of the 12 lots. The ash was included in another 4 diets at a level of 0.8 to 0.9%, equivalent to the amount of ash supplied by the beet pulp added. Ash was obtained by first collecting the ash of beet pulp burned in 50-gallon drums. This ash was then placed in a muffle furnace for 15 minutes at 600°C. In the remaining lots neither beet pulp nor its ash was supplied. Sodium chloride was included at 0.5% in half of the diets and at 10% in the other half. The 10% level of salt was arbitrarily selected to furnish a large salt intake, without restricting feed consumption. Potassium acid phosphate was added to half of the diets at 11 g per lb of diet, the level reported to increase the occurrence of urinary calculi(1,2). Lambs were observed daily to detect symptoms. When noticed, the lamb was sacrificed and autopsied. At conclusion of the experiment, the remaining animals were

TABLE II. Incidence of Urinary Calculi by Treatment.*

NaCl, %	No beet pulp		Ash of beet pulp		Beet pulp		Total
	Control	K_2HPO_4	Control	K_2HPO_4	Control	K_2HPO_4	
10	0	0	0	0	0	0	0
.5	0	4	1	4	2	3	14

* 5 sheep on each treatment.

slaughtered and the entire urinary tract of each was removed and examined for calculi. Those having stones present at slaughter were included as calculi cases along with the lambs already autopsied. Calculi recovered from the urinary tracts were not chemically analyzed in this experiment. Calculi in sheep receiving similar rations as fed in this experiment were reported to contain principally calcium and magnesium phosphate(1,2).

Results. During the 105 days of the observation, 10 animals displayed symptoms of urinary calculi, with the first occurring on 33rd day. The symptoms observed were anorexia, restlessness, and straining in an attempt to urinate. The afflicted lambs often stood with their hind feet placed far to the rear and frequently shifted their weight on their rear legs. At the time of autopsy, the urinary tract was usually damaged extensively. The bladder was distended severely and kidneys were often in a state of acute hydronephrosis. Calculi were found in the bladder in almost every instance, but in only 2 cases were stones found in the kidneys. The site of occlusion of the urinary tract with calculi was usually the urethral process.

At the conclusion of the experiment, when the remaining 50 lambs were slaughtered, 4 more lambs had calculi in their urinary tracts. Thus, a total of 14 cases of calculi developed during the experiment (Table II).

Addition of 10% sodium chloride to the diets of 30 sheep prevented occurrence of urinary calculi. No decrease in feed consumption was observed in the lambs receiving this high level of salt. However, they did void considerably more urine as evidenced by the condition of the litter. The 14 cases of calculi that resulted were in the 30 sheep receiving 0.5% of added sodium chloride in their diets.

Of the sheep which received the lower level of sodium chloride in their ration, 15 also received the potassium acid phosphate supplement. Eleven of these animals developed calculi, while 3 of the 15 not receiving potassium acid phosphate formed calculi. Thus, 11 of the 14 cases of urinary calculi occurred in sheep fed the 0.5% level of sodium chloride plus potassium acid phosphate. This follows closely the results of previous studies in which potassium acid phosphate caused an increased incidence of calculi.

The addition of the ash of beet pulp or of beet pulp itself to the diet did not alter the incidence of calculi. There were 5 cases produced, both when the ash was added and when beet pulp itself was added. Four cases occurred in the lots receiving neither of the supplements. These results do not agree with those of previous studies in which beet pulp increased the incidence of calculi. The reason for this lack of agreement is unknown. Apparently the beet pulp varied enough in composition from that used in the previous experiments to cause this difference in results. It is interesting to note that the 3 sheep that developed calculi in the absence of added potassium acid phosphate were receiving either beet pulp or its ash.

Discussion. The reason for the prophylactic effect of the high level of sodium chloride in the diet is not known, but there are several possible explanations. Perhaps the diuresis resulting from feeding a diet high in salt is sufficient to wash any stones which may form out of the urinary tract before their size is great enough to block urine passage. On the other hand, the prophylaxis may be due to inhibition of calculi formation. The increased volume of urine voided may dilute the crystalloids present to such an extent that precipitation is prevented, or at least hindered.

Since sheep urine is normally alkaline in reaction the preventive effect may have been mediated through a decrease in urine alkalinity. Investigations with rats(5) and mink (6) have indicated that the addition of ammonium chloride to the diet prevented urinary calculi formation. Leoschke and Elvehjem (6) reported that the addition of one gram of ammonium chloride daily to a diet fed to mink reduced the incidence of urinary calculi. They attributed the effect to the increase in urinary acidity. Feeding sheep a 10% level of sodium chloride in diets similar to those fed in this experiment has resulted in an increase in urinary acidity from an average pH of 7.95 to 7.30.† Using analysis of variance, this difference was statistically significant (P <.01). The urine pH was determined by collecting a 24-hour urine sample under toluene and immediately measuring the pH of the urine with a glass electrode pH meter.

The effect of the high salt diet may be related to the relationship which exists in the body between sodium, potassium and other minerals. Of course, a combination of several or all of the above mentioned factors may have been responsible for the preventive effect of the 10% level of sodium chloride. Whatever the reason, it should be emphasized that additional investigation is needed before the use of a high sodium chloride diet to prevent urinary calculi can be recommended.

† Elam, C., and Ham, W., unpublished data.

Summary. Sixty wether lambs were divided into 12 lots of 5 each and fed the experimental diets for a 105-day period. The animals were group fed all they would consume twice daily. The variables included in the diets were the presence and absence of potassium acid phosphate (K_2HPO_4), a 10% level of sodium chloride, a 30% level of beet pulp, and the ash of the same amount of beet pulp. Fourteen cases of urinary calculi resulted in the 60 sheep. None of these cases occurred in the 30 sheep which received the 10% level of sodium chloride. Eleven of the 14 cases of calculi occurred in those lots receiving added potassium acid phosphate in the diet, while 3 occurred in its absence. Beet pulp or beet pulp ash had no effect on the incidence of urinary calculi.

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Effect of Vitamin D on Retention of Radium by the White Rat.* (23360)

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The effect of vit. D on retention of radium in white rats was studied as part of a general investigation on distribution and behavior of radium in the animal body. It has been believed since the early studies on radium poisoning that radium is deposited and retained in the skeletal structure. Evans *et al.*(1), using radioautographs, confirmed this belief, showing that radium was concentrated in the region of the epiphyses. Norris and Kisieleski (2) found that 3 days after intravenous administration of radium skeletal retention was 63% of the injected dose; retention was still 53% after 30 days. Since total body retention after the 30 days was 55% of the injected dose, practically all the radium re-

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