mouse serum was added to the 1% suspension, good concentration occurred and was associated with the appearance of a discrete pellet. The results of this study did not permit conclusions as to the mechanism involved and the variable factors in the system were not further studied.

A disadvantage of calf serum for concentration is the too frequent presence of inhibitors to poliovirus. One alternative was to substitute sera from other species. Results with horse and rabbit sera indicated that these sera at 3% concentration lacked the ability to form a similar enmeshing pellet. Electrophoretic studies of calf serum pellets showed that they were mainly composed of the larger serum molecules. This suggested that other large protein molecules would form such a pellet. A study of some readily available proteins showed that gelatin (Nutritional Biochemicals Corp.) could form a poliovirus-enmeshing pellet and was free of inhibitors.

The data presented demonstrate that reproducible concentration of poliovirus can be accomplished by sedimentation at 78,000 times gravity in the presence of 2% inhibitor-free calf serum or 0.06% gelatin. Such concentrated virus can be used for chemical, physical and serological studies as well as safety testing of larger volumes of poliomyelitis vaccines in a simplified, practical manner.

Summary. Studies were undertaken to determine the efficiency of concentration of poliovirus from tissue culture fluids in the ultracentrifuge. Under suitable sedimentation conditions most of the virus was concentrated to the lower portion of the centrifuge cup, but in the absence of calf serum was not present in a sediment adherent to the cup wall. Addition of calf serum resulted in formation of a firm, discrete pellet attached to the centrifuge cup wall which contained more than 95% of the virus. Further experiments indicated that the activity was associated with the globulin protein fraction of calf serum and that gelatin had a similar effect. Studies varying the amounts of calf serum and gelatin and duration of ultracentrifugation indicated that 2% calf serum or 0.06% gelatin and 3 hours centrifugation are minimal for effective concentration of poliovirus when sedimented at 78,000 times gravity in the number 30 rotor of the Spinco model "L" preparative Ultracentrifuge.

The author would like to acknowledge the valuable suggestions made by Dr. Robert Rustigian during the initial stages of this study, and the technical assistance of Mr. George Gardner.

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Received June 25, 1957. P.S.E.B.M., 1957, v95.

Postirradiation Creatinuria in *Macaca mulatta*.* (23357)

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The authors have observed a profound creatinuria in rats immediately following large doses of radiation(1). Subsequently it was found that 2 dogs irradiated with 400 and 500 rep of X-ray, respectively, did not excrete ex-

* This work was supported in part with funds provided under Contract AF 41(657)-149 and the School of Aviation Medicine.

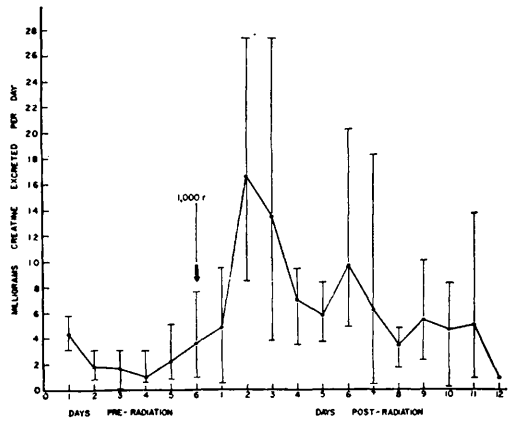

FIG. 1. Daily urinary creatine excretion of 4 male adult monkeys before and after 1,000 rep of X-irradiation. Each circle represents the mean and the barred lines represent the extremes excreted.

cessive amounts of creatine in the urine immediately following irradiation(2). In this paper we wish to report evidence for postirradiation creatinuria in the monkey.

Materials and methods. Four normal adult male *Macaca mulatta* monkeys approximately 3 to 4 years old, weighing an average of 10 lb, were placed in metabolism cages and fed a creatine-free diet(1). After stabilization on the diet for 7 to 9 days, the animals were anesthetized with 10 mg/lb of Nembutal and were irradiated with a Picker X-ray machine at the rate of 75 r/min. for a total dose of 1,000 rep. Constants of the machine were 250 KVP, 18 Ma, filter 1.0 mm Al plus 0.25 mm Cu at a distance of 60 cm from target to object. Dosimetry in air was performed with a Victoreen rate-meter which had been previously calibrated against a source from the National Bureau of Standards. Urinary creatine determinations were made by the method of Anderson *et al.*(3).

Results. Two of the animals excreted large amounts (19.7 and 26.2 mg, respectively) of creatine on the second day of the diet. By the third day of the diet, however, all animals had become stabilized to a consistently low level of urinary creatine excretion. The average daily excretion for the 4 animals for the 6 days prior to irradiation was 2.5 mg. The average excretion was approximately twice normal on the first postirradiation day, 7 times normal on the second postirradiation day, and 5 times normal on the third postirradiation day (Fig. 1). A secondary creatinuria occurred on the sixth day when the average was approximately 4 times normal. One animal died on the eighth, 2 on the twelfth, and one on the thirteenth postirradiation day.

Discussion. The results in these 4 monkeys are clear: a postirradiation creatinuria about 7 times normal occurred in all animals. This picture is very similar to that found in the rat(1). The terminal creatinuria which we have observed in most of our rats occurred in one monkey (15.8 mg on the last day of life), but not in the other 3.

Zhahova and Broun(4) state that postirradiation creatinuria was first observed in man in 1938 and was subsequently confirmed by experiments on dogs. Unfortunately, no references are given for these statements, nor are any of the details as to dose, magnitude of effect, etc., discussed. These authors do, however, publish figures for postirradiation creatinuria in 90 rats. An approximate 4- to 5-fold creatinuria on the second day followed an acute dose of 500 rep of X-rays. Haberland *et al.*(5) report an approximate 2-fold creatinuria on the second postirradiation day following 759 r of X-ray in rats. However, the control values for daily creatine excretion are higher than any we have ever encountered, and since these animals were not fed a creatine-free diet prior to radiation, it is possible that the experiments are not entirely comparable to our own. The discrepancy between our results for the dog(2) and those reported by Zhahova and Broun may be due to a difference in dose administered. Some of the details of previously published work are given in Table I.

Summary. Four adult male *Macaca mulatta* monkeys weighing an average of about 10 lb were placed on a creatine-free diet for 7 to 9 days. After the urinary level of creatine excretion had reached a plateau, the animals were irradiated with 1,000 rep of X-rays. A creatinuria of approximately 4 times normal was excreted over the first 4 days in a pattern similar to that previously found in the rat. On the second postirradiation day, the

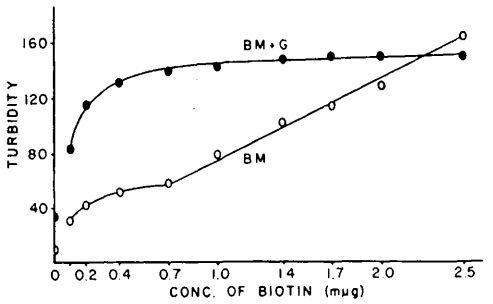

FIG. 2. Effect of inclusion of glucose in growth medium on response of *E. coli* to biotin. 36 hr incubation at 30°C under stationary conditions. BM = Basal medium. BM + G = Basal medium + 1% glucose.

shown in Fig. 2. The most striking effect was the marked sparing action of glucose on the biotin requirements of this organism. This sparing effect was noted not only at sub-optimal levels of biotin but was reflected also in the absence of added biotin (*i.e.*, the tubes containing basal medium plus glucose but without added biotin routinely supported 3-5 times as much growth as identical tubes without glucose). This effect of glucose was quite surprising and the mechanism is still under investigation. The increased growth in the presence of glucose does not appear to be related to biotin synthesis since microbiological assays of acid hydrolyzed cells with *Saccharomyces cerevisiae* (139) as the assay organism revealed the same biotin content for cells harvested from media without added biotin in the presence or absence of glucose. Furthermore, avidin did not antagonize this effect of glucose when incorporated into the growth medium.

The possible use of this biotinless mutant strain of *E. coli* for microbiological assay of biotin would appear to be limited to samples contributing amino acids and/or glucose in amounts less than that required to produce the disturbing effects discussed above. It is probable therefore that use of this organism as an assay tool will be limited to research problems with relatively pure samples.

It is well established that incorporation of certain fatty acids, particularly the 18-carbon unsaturated members, reduces the requirement for biotin by lactic acid bacteria(4). It was desirable therefore to ascertain the effect, if any, of fatty acids on response of *E. coli* to biotin. This was determined by using oleic and lauric acids in the form of Tween 80 and 20 respectively (Table I). The results reveal that under stationary conditions of incubation both Tween 20 and 80 at a concentration of 10 μg/tube caused a stimulation of growth at sub-optimal levels of biotin. No increase in total growth was noted and non-specificity was suggested by the fact that both Tweens worked equally well. At higher concentrations (100 μg/tube) definite inhibition of growth was noted, which is in keeping with observations with other microorganisms. It is pertinent that the Tweens had no effect on growth of this organism in the complete absence of biotin which agrees with the recent work of Traub and Lichstein(5).

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	Turbidity				
	Biotin (mμg/tube)				
Additions	None	.1	1.0	3.0	5.0
None	2*	25	106	199	224
Tween 80, 10 μg	4	38	153	234	226
" ", 100	3	32	57	68	73
Tween 20, 10	2	37	158	238	222
" ", 100	3	38	58	72	79

* Klett-Summerson readings after 36 hr incubation at 30°C.

The data presented in Table II reveal the stimulatory effect of CO_2 on growth of *E. coli* incubated under stationary conditions in the presence of biotin. Although the interpretation of these results is of necessity limited, it

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2.0	126	201
2.5	164	210
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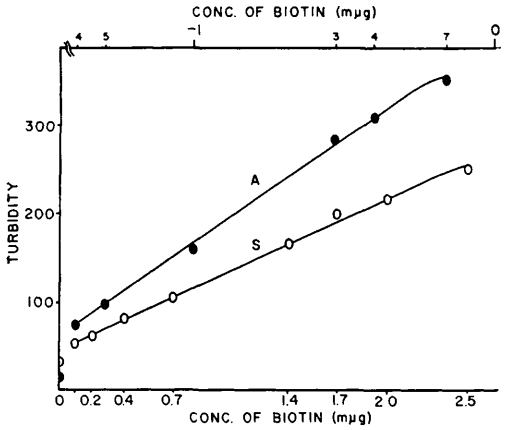


FIG. 1. Response of *E. coli* to biotin. 36 hr incubation at 30°C. S = Stationary incubation (arithmetic scale). A = Aerobic incubation (logarithmic scale).

less and the volumes adjusted to 1 ml with distilled water. The tubes were sterilized by autoclaving for 15 min. at 121°C. After cooling, 4 ml of previously inoculated medium (1 drop of inoculum diluted 1:10 per 100 ml of sterile medium) were added to each tube. The contents were mixed by shaking and incubated at 30°C for 30-36 hrs. For aerobic cultivation the assay vessels were 25 x 150 mm tubes fitted with one hole rubber stoppers plugged with cotton, and incubation was carried out on a reciprocal shaking machine.

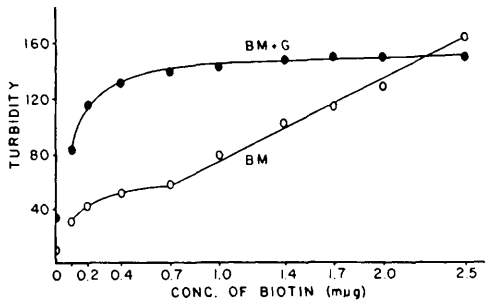
Growth response was determined turbidimetrically with a Klett-Summerson photoelectric colorimeter equipped with a blue filter (400-450 mμ). Prior to reading, each tube was shaken vigorously to suspend the cells and the instrument set at zero with a blank containing 4 ml of uninoculated medium and 1 ml of water.

Results. Typical growth responses of this organism to varying concentrations of biotin under stationary and aerobic conditions of incubation are given in Fig. 1. It is to be noted that the dose-response curve of this organism to biotin appears to depend on degree of aeration during cultivation. Thus, a linear turbidity increase with arithmetic increases in biotin concentration was obtained under stationary conditions of cultivation, while a straight line response was obtained under aerobic conditions of incubation only when the log concentration of the vitamin was employed. The linear response to logarithmic increases in concentration of biotin is typical of most microorganisms employed for assay of this vitamin (*i.e.*, species of *Saccharomyces* and *Lactobacillus*), whereas the straight line response to arithmetic concentrations of biotin has been reported previously only for propionibacteria(2). For assay purposes, the usable range of response extends from 0.1-2.5 mμg of biotin for stationary cultures and from 0.04-0.7 mμg for aerated cultures. Linearity was maintained below the indicated lower limits, however in this range relatively large changes in vitamin concentration produced small differences in turbidity.

The next phase of the study was an investigation of the influence of certain nutritional factors on response of this strain of *E. coli* to biotin. For this study stationary conditions of incubation were employed.

Inasmuch as biotin has been implicated in certain areas of nitrogen metabolism, it appeared desirable to inspect the effect of concentration of amino acids in the growth medium on the response of *E. coli* to biotin. It was found that 0.5% casamino acids in the medium was optimal for the response of this microorganism to biotin considering range of linear response, sensitivity and reproducibility of results. At low concentrations of casamino acids (0.1%) growth was limited and range of response to biotin was quite narrow as might well be expected since the amino acids serve as the principal source of energy in the absence of added carbohydrate. High concentrations of casamino acids (1-2%) resulted in marked inhibition of growth at low levels of biotin, and this inhibition was overcome by the presence of optimal amounts of this vitamin. It is possible that growth inhibition may have resulted from the inefficient metabolism of some amino acids due to a deficiency in biotin-linked reactions with an accumulation of such amino acids in toxic amounts. The adverse effect of amino acids on bacteria under certain conditions is well recognized and was reported first by Gladstone(3) working with *Bacillus anthracis*.

The effect of inclusion of glucose in the medium on biotin requirements of *E. coli* is



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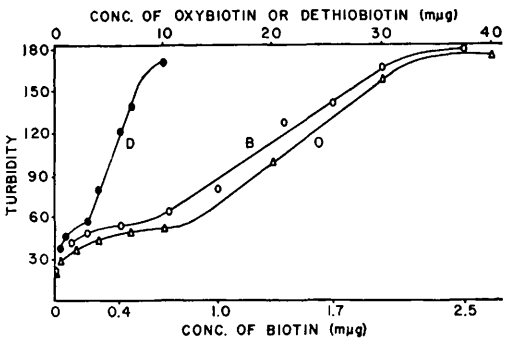

FIG. 3. Response of *E. coli* to biotin, oxybiotin and dethiobiotin. 22 hr incubation at 30°C under stationary conditions. B = D-biotin. O = DL-oxybiotin (o—heterobiotin). D = DL-dethiobiotin.

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.

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.

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

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Received June 26, 1957. P.S.E.B.M., 1957, v95.

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

C. J. ELAM, W. E. HAM, AND B. H. SCHNEIDER
(Introduced by James McGinnis)
Department of Animal Science, State College of Washington, Pullman

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-

* Scientific Paper no. 1524. Washington Agri. Exp. Station, Pullman. Project No. 1001. Presented by C. J. Elam as part of thesis in partial fulfillment of requirements for Ph.D. degree.

This investigation was supported in part by funds provided for biological and medical research by State of Washington Initiative Measure No. 171.