Table 1. Effect of clarified rumen fluid, fractions of rumen fluid, and known volatile fatty acids on growth of cellulolytic cocci from the bovine rumen.

Addition to basal medium	Growth of strain (optical density × 100)†				
	7	20	FD-1	B146	C94
	*Experiment 1**				
No addition	0(168)	0(168)	11(123)	14(168)	0(168)
Clarified rumen fluid	69(15)	78(15)	64(15)	72(19)	64(19)
Acid steam distillate	67(19)	83(19)	34(47)	37(30)	53(24)
Residue from acid steam distillate	0(168)	0(168)	16(100)	14(71)	15(168)
Acid steam distillate + residue	70(15)	69(15)	50(19)	63(30)	37(24)
	Experiment 2‡				
Acetate	0(168)	0(168)	12(168)	13(168)	0(168)
Acetate, propionate, *n*-butyrate, isobutyrate, *n*-valerate, isovalerate, DL-α-methyl-*n*-butyrate, and *n*-caproate	75(24)	63(24)	42(48)	32(24)	53(30)
Acetate, isobutyrate, *n*-valerate, isovalerate, and DL-α-methyl-*n*-butyrate	79(24)	66(30)	47(42)	40(19)	60(24)
Acetate, propionate, *n*-butyrate, and *n*-caproate	5(120)	9(120)	13(48)	10(120)	0(168)
Acetate and isovalerate§	64(24)	57(30)	49(42)	29(42)	54(36)

* Levels added were 8 percent of clarified rumen fluid or the amount of the fraction equal to 8 percent clarified rumen fluid.
† The number in parentheses is the number of hours of incubation required to reach maximum growth.
‡ The levels of fatty acids added were the following in millimoles per 100 milliliters: acetate, 4; propionate, 0.13; *n*-butyrate, 0.065; isobutyrate, *n*-valerate, isovalerate, and DL-α-methyl-*n*-butyrate, 0.0128; and *n*-caproate, 0.0064.
§ The level of isovalerate was increased to 0.5 m mole/100 ml.

replaced the cysteine in the basal medium, increased growth was obtained. The precipitate which formed when sulfide was added was eliminated by exclusion of $FeSO_4$, $ZnSO_4$, $CaCl_2$, and $CoCl_2$. This modified basal medium, containing 50 mg of $Na_2S \cdot 9H_2O$ per 100 ml, was used in subsequent experiments and permitted growth with fatty acids approaching that obtained with clarified rumen fluid.

The volatile acid fraction of clarified rumen fluid obtained by steam distillation at *p*H 1.5 supported growth of strain C-94 similar to that obtained by an equivalent concentration of rumen fluid, while the nonvolatile residue did not support growth.

To determine whether other strains of cellulolytic cocci require volatile fatty acids, strains FD-1 and B_1-46 similar to *Ruminococcus flavefaciens* and strains 7 and 20 of *R. albus* (*1*) were studied.

The effect of known volatile fatty acids and fractions of clarified rumen fluid obtained by steam distillation on growth of the five strains is shown in Table 1. All of the strains were markedly stimulated by the steam distillate while the residue contained little or no growth promoting factor(s). The mixture of eight known volatile fatty acids stimulated growth of all strains, as did a mixture of acetate, isobutyrate, and the valeric acid isomers and also a mixture of acetate and isovalerate. A mixture of acetate, propionate, *n*-butyrate, and *n*-caproate contained little growth promoting activity.

The fatty acid growth factor for the cellulolytic cocci appears to be different from that required by the ruminal cellulolytic bacterium, *Bacteroides succinogenes* (*2*). Isovalerate stimulates growth of the cocci, while *B. succinogenes* requires a straight-chain acid such as *n*-valerate or *n*-caproate as well as a branched-chain acid. The factor is also distinct from that required by the *Ruminococcus albus* strain 69 of Fletcher (*4*), which requires a nonvolatile acidic component of rumen fluid. Since all the strains in the present study were greatly stimulated by volatile fatty acids and were isolated from three animals, from two locations, on four different rations, and were selected as representative of both ruminococcus species, it is suggested that organisms with similar nutritional characteristics are quite numerous.

It is now known that strains of three species of bacteria which appear to be among the most numerous and most active cellulolytic bacteria in the bovine rumen require or are greatly stimulated by volatile fatty acids. These findings exemplify the interdependence of rumen microorganisms since the branched-chain fatty acids required by these bacteria are apparently produced from amino acids by other microbial species of the rumen (*5*).

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26 March 1958

Inhibition of Postirradiation Diuresis in Rats by Pitressin

Many observers have reported that total-body irradiation results in an immediate polydipsia and polyuria in the rat (*1–3*). Recent experiments in this laboratory have shown that the thirst and diuresis may be prevented by injection of the antidiuretic hormone after irradiation (*4*).

Fifty-four rats (four adult Sprague-Dawley females and 50 adult Holtzman females) weighing between 150 and 200 g were selected at random from a holding colony. Twenty rats served as controls. The remainder of the rats were irradiated with 1500 r in a Co^{60} facility (*5*) at the rate of 1500 r/min. Within 1 minute after irradiation, the animals were injected intramuscularly by means of a tuberculin syringe and a 22- or 23-gauge needle. Nineteen animals were injected with 500 milliunits of Pitressin tannate (*6*) in 0.1 ml of peanut oil. Fifteen animals received 0.1 ml of peanut oil (*7*). The animals were then placed in individual metabolism cages and allowed free access to tap water but were deprived of food. Water intake and urine output were measured at 24 hours. The results are shown in Table 1.

The irradiated, peanut-oil injected animals exhibited a significant polydip-

Table 1. Average water intake and urinary output per animal during the first 24 hours after 1500 r of Co^{60} irradiation. The number of animals is given in parentheses. All injections were made immediately postirradiation.

Treatment	Water intake (ml)	Urinary output (ml)
Control: fasted, nonirradiated, noninjected animals (20)	10 ± 9	9 ± 5
Irradiated, fasted animals injected with 0.1 ml of peanut oil (15)	55 ± 24	47 ± 21
Irradiated, fasted animals injected with 500 milliunits of Pitressin in 0.1 ml of peanut oil (19)	16 ± 7	7 ± 3

sia ($P < .001$) and a significant polyuria ($P < .001$) compared with the polydipsia and polyuria of the nontreated controls or of the irradiated, Pitressin-injected animals. The urinary output values of the irradiated Pitressin-injected animals were not significantly different from the outputs of the nontreated control animals, but the water intakes were significantly higher than the intakes of the nontreated controls ($.02 < P < .05$). Water intakes and urine outputs within experimental groups were not significantly different in the case of the nontreated controls or the irradiated peanut-oil injected animals. However, water intakes were significantly greater than urine outputs ($P < .001$) in the irradiated, Pitressin-injected group.

These results show that postirradiation polyuria is not due to an obligatory renal tubular water loss, but they do not offer proof for any of the postulated mechanisms. If the phenomenon is the result of an imbalance between adrenal cortical diuretic and posterior pituitary antidiuretic factors as proposed by Smith and Tyree (*2*), the evidence in this paper would suggest that the imbalance can be corrected by the injection of Pitressin.

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24 February 1958

Quantitative Roentgenography of Skeletal Mineralization in Malnourished Quechua Indian Boys

Roentgenograms of the hand suitable for densitometric analysis as well as assessment of skeletal age were taken on 91 Quechua Indian boys from Hacienda Vicos in the North Central Peruvian Sierra. These boys were from a biologically isolated inbreeding population of over 1800 Indians almost wholly unmixed with Europeans (*1*). This Indian population lives in a narrow upland valley 10,000 to 12,000 feet in altitude, off the Callejon de Huaylas 250 miles north of Lima. Until 1957 the Vicos Indians were economically depressed serfs exploited by a long series of absentee landlords, but then they were freed by government proclamation.

The principal Vicos food crops are corn and potatoes; intake of animal proteins is negligible. Dietary surveys in 1952 and 1953 (*2*) and a check study in 1956, when the roentgenograms were taken, revealed that the average daily intake of vitamins A and B_2 and calcium were respectively 3, 56, and 14 percent of adjusted INCAP recommended allowances (*3*). Preliminary studies suggest that Vicos soils are calcium-deficient. Calorie and protein intake were about 70 percent of the recommendations, and fats represented only 7 percent of the daily caloric intake. Superposed on this inadequate nutrition was a heavy infestation of intestinal parasites, particularly *Ascaris* (*4*).

The posteroanterior roentgenograms of the left hand were taken by Humberto Mesones, through the courtesy of Ramon Vallenas, subdirector of Peru's Department of Industrial Hygiene. The roentgenographic technique, the developing procedure, and the photodensitometric apparatus used in the analysis have been described elsewhere (*5*) and need only brief mention. Eight- by ten-inch No-Screen film in cardboard holders was used, and an aluminum-zinc alloy film calibration wedge was simultaneously exposed with the subject's hand. The x-ray apparatus was a mobile Phillips 15 M A, manufactured in the Netherlands. Electric current was provided by a 220-v 60-cy/sec gasoline generator with a 3500-watt output. Exposure time was lengthened slightly to compensate for drawdown. The developed film was placed in the photodensitometric apparatus, and the middle phalanx of the fifth finger was evaluated for bone density coefficients at three sites or trace paths: the proximal and distal ends, whose values were averaged, and the centers. In this analysis the bone density coefficient is the number of grams of the aluminum-zinc alloy which, in a similar geometric shape, would absorb the same amount of x-radiation as the bone, divided by the volume of bone in cubic centimeters.

The Quechua Indian means, grouped by age (Table 1), stand in decided contrast to a series of normal white boys drawn from various parts of the United States (*6*). The means for the 7–9- and 10–12-year-olds of both Indians and whites are similar, but then the 13–15- and 16–20-year-olds from the United States show increases statistically significant at the .001 level, or better, for

Table 1. Mean density coefficients of the phalanx center and phalanx end trace paths of Vicos Indian boys and United States white boys.

Age group	Cases (No.)	Density coefficient (mean ± standard deviation)	
		Phalanx center	Phalanx end
		91 Vicos boys	
7–9	18	0.98 ± 0.30	0.59 ± 0.16
10–12	29	1.06 ± 0.23	0.65 ± 0.15
13–15	31	1.02 ± 0.21	0.64 ± 0.14
16–20	8	0.99 ± 0.40	0.66 ± 0.25
		624 United States white boys	
7–9	77	1.02 ± 0.23	0.64 ± 0.11
10–12	101	1.03 ± 0.28	0.66 ± 0.16
13–15	225	1.32 ± 0.30	0.89 ± 0.21
16–20	221	1.43 ± 0.24	1.03 ± 0.19

both the phalanx end and center trace paths. No such increase is present in the Indian boys in the age groups studied. The mean differences between Indians and whites in the 13–15- and 16–20-year groups are also statistically significant at the .001 level for each trace path. Accordingly, bone mineralization shows steady mean increases through the teens in United States whites but none at all in the Indians.

The lack of increased bone density through the late teens in the Vicos Indians is probably due to dietary inadequacies. This view is supported by Williams and associates (*7*), who observed that bone-density measurements reflected the nutritional history of an individual. In addition, radical decreases in dietary calcium in rats depress bone-density values of their femurs (*8*) and caudal vertebrae (*7*). While this suggests that the depressed bone density coefficients of the Vicos Indians may be a result of their low calcium intake, the marked deficiencies in other nutrients and the extreme parasite infestation must also be considered. At Vicos, augmented bone mineralization, indicated by higher density values, may occur in the early 20's although we have no roentgenograms of this age group. The higher standard deviations in the numerically inadequate 16–20-year-old group may presage such an increase in bone mineralization. Indeed, the chewing of coca by almost all the Vicos Indian men (but never by the women) may result in increased calcium ingestion, since release of the alkaloid in coca is accomplished by mixing *cal* with it. *Cal* is thought to contain considerable quantities of calcium and is the Spanish word for lime.

The depressed bone mineralization in the Vicos boys is paralleled by an average lag of several years in skeletal ma-