in a unit which bears a simple relationship to dose. It was noted that the combined plot had the general appearance of a sigmoid curve and should therefore be approximately linear on probit transformation (Fig. 1*B*).

In order to test the general applicability of this procedure, control data from all other experiments with the same strain and sex of mice and the same radiation factors (*4*) were compiled and plotted as shown in Fig. 2. Data for survival time could be used for the groups in which fewer than 50 percent survived. The results are essentially the same as those shown in Fig. 1.

A general procedure, therefore, for converting the two responses to the same unit is obtained by defining an effect W (*5*), such that

$$W = \frac{p+1}{2}$$

$$= \frac{D-4}{2(D_0-4)}$$

where p is the proportion surviving, D is the mean survival time, and D_0 is the mean survival time of mice given the minimum radiation required for approximately zero survival.

For many purposes in which graphic analysis is adequate it is sufficient to use the left-hand ordinate for percentage survival and the right hand ordinate for survival time, with the latter so scaled that at zero survival $D = D_0$ and at 100 percent survival $D = 2D_0 - 4$. If a linear dose-response curve is desired W may be plotted on probit paper, but the usual maximum likelihood calculation procedure is not applicable because the standard error of W is not the same as the standard error of an equal p.

The standard errors shown in Fig. 2 are estimated from the variation of results from experiment to experiment rather than from the pooled internal variation. The points representing percentage survival and survival time at the same dose level fall on essentially the same curve. Although some increase in precision could be obtained at these levels by combining the two responses, the simplicity of the procedures involved in using percentage survival alone has much to recommend it.

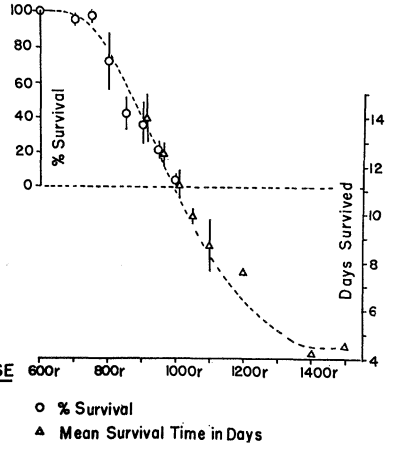

Fig. 2. Plot of percentage survival (open circles) and mean survival time (open triangles) by radiation dose for 66 control groups of mice not represented in Fig. 1.

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4. Mice were (BALB/c × DBA/2)F_1 females 12 to 15 weeks old, kept in individual cages. Irradiation was from a Van de Graaf generator operating at 2.5 Mev, 0.6 HVL 1 cm lead, TSD 1 m, and dose rate 250 to 300 r/min. We are indebted to Dr. Howard L. Andrews for dosimetry and irradiation.
5. For Waldorf.

14 April 1958

Volatile Fatty Acid Growth Factor for Cellulolytic Cocci of Bovine Rumen

The anaerobic cellulolytic cocci belonging to the genus *Ruminococcus* (Sijpesteijn) Hungate (*1*) are considered to be important participants in rumen fermentation because of the large number that are present in the rumen and because of their capability for rapid digestion of cellulose. These bacteria comprise one of the groups of rumen organisms that require or are stimulated by a factor(s) present in rumen fluid which is not usually detectable in the usual ingredients of bacteriological media. Strain C-94, a representative of *Ruminococcus flavefaciens* isolated from 10^{-8} ml of bovine rumen contents, was chosen for this study to determine its requirement for a rumen fluid factor(s).

A basal medium was developed which contained the following substances (in milligrams per 100 milliliters): thiamin · HCl, Ca-D-pantothenate, riboflavin, and nicotinamide, 0.2; pyridoxamine · 2HCl, pyridoxal · HCl and pyridoxine · HCl, 0.1; *p*-amino benzoic acid, 0.01; biotin and folic acid, 0.005; cobalamin, 0.0005; casein hydrolyzate (acid), 200; Tween 80, 4; cellobiose, 300; resazurin, 0.1; Na_2CO_3, 400; KH_2PO_4, NaCl, and NH_4SO_4, 90; $CaCl_2$, $ZnSO_4$, $MgSO_4 \cdot 7H_2O$ and $MnSO_4 \cdot H_2O$, 1; $FeSO_4 \cdot 7H_2O$, 2; $CoCl_2 \cdot 6H_2O$, 0.4; and cysteine · HCl · H_2O, 100. The medium was adjusted to *p*H 6.7 and was sterilized and inoculated under CO_2 by the anaerobic technique of Hungate as used by Bryant and Doetsch (*2*). Additions to the basal medium were separately sterilized and were combined after autoclaving.

Growth was expressed as optical density (OD) as determined with a Bausch and Lomb Spectronic 20 colorimeter at 600 mμ. Inoculum was prepared from a 24-hr culture in a medium containing 20 percent clarified rumen fluid (CRF), and minerals, cellobiose, resazurin, and reducing agent as in the basal medium. The culture was centrifuged, the supernatant was decanted, and the cells were diluted to an optical density of 0.1 with the anaerobic dilution solution of Bryant and Burkey (*3*). Five-milliliter volumes of medium were inoculated with 0.1 ml of the cell suspension. The clarified rumen fluid consisted of supernatant after fresh rumen fluid was centrifuged at 25,000 *g* for 30 minutes.

The basal medium did not support growth of strain C-94 alone or when the following were added: enzymatically hydrolyzed casein; purines and pyrimidines; inositol, choline and sodium acetate; or glutamine, coenzyme I, and glutathione. Clarified rumen fluid and bovine feces extract supported good growth. Polypeptone, trypticase, thiotone, and lactalysate each supported growth in the basal medium, but growth was much delayed compared with that in clarified rumen fluid or feces extract. Crude materials incapable of supporting growth when they were added to the basal medium included peptone, phytone, yeast extract, beef extract, liver extract, corn steep water, distillers' dried solubles, and a hot-water extract of a mixture of alfalfa and brome grass.

It has been shown that certain volatile fatty acids present in rumen fluid are required by a rumen bacterium (*2*). A mixture of known acetate, propionate, *n*-butyrate, isobutyrate, *n*-valerate, isovalerate, DL-α-methyl-*n*-butyrate, and *n*-caproate in proportions similar to those found in rumen fluid supported growth of strain C-94 when it was added to the basal medium. Deletion of acetate from the mixture resulted in a marked increase in the incubation time required to reach maximum growth. When the other acids were added singly in the presence or absence of acetate, only isovalerate and isobutyrate promoted growth. Acetate shortened the lag phase of growth when it was added with these acids. No effect on growth was noted when *n*-valerate was added to the basal medium plus acetate and isobutyrate and/or isovalerate.

Growth in the basal medium with added fatty acids was much less than it was when clarified rumen fluid was present. During a study with various reducing agents it was found that, when Na_2S

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, non-irradiated, 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