vestments: the large counter in operation in Groningen requires 8 liters of CO_2. A sample enriched 16 times contains as much C^{14} as 128 liters of the original material. Since the depletion of the reservoir should be at most about 30 percent in order to avoid an appreciable decrease in the transport of C^{14}, about 350 liters of sample are required at the least.

The enrichments are performed at the Laboratory for Mass Spectrography in Amsterdam. The CO_2 obtained from the original material (*1*) is reduced to CO by leading it over zinc at a temperature of 380° ± 5°C. The CO is enriched in five thermal diffusion columns connected in parallel, each of them having a length of 430 cm. The enrichment is derived from the O^{18} abundance in the CO (*2*). For the activity measurements, the enriched CO is oxidized again by leading it over CuO. Enrichment by a factor of 16 takes at present about 2 months; this enrichment shifts the limit of counting to about 70,000 years.

Up to now three samples have been measured. The first sample consisted of recent carbon dioxide diluted with a known amount of inactive CO_2. The enrichment predicted by the abundance of O^{18} was 8.70 ± 0.5; the enrichment measured was 8.06 ± 0.05.

The second sample consisted of dead CO_2 (from anthracite). If all steps are completely free from contamination by recent carbon, the enriched sample should still be inactive. The activity was actually 0.04 ± 0.02, corresponding to an apparent age of 73,000 years. The activity is not really significant, however. This experiment will be repeated in due course.

The third sample consisted of wood from a well-exposed profile at Amersfoort (Netherlands). The situation has been described in more detail by H. de Vries (*3*); it is indicated as Amersfoort XII. The present sample dates the end of the Last Interglacial or an early Würm interstadial separated from the Last Interglacial by a not very cold period. The age found by enrichment was 64,000 ± 1100 years (sample Gro-1397). The limits of error include the statistical error only. Errors introduced by the enrichment and measurement of the enrichment amount to about 800 years. The most important source of error, however, for these old samples is infiltration of recent material (see also *3*). The wood had been cleaned thoroughly by chemical treatment. Humus extracted from it has an age of 42,300 ± 900 years only. So even this wood, protected by a shield of compressed peat and 9 meters of sand, had acquired some recent hume (the extracted humus is a mixture of original material and infiltrated material). Because of possible infiltration, all dates are more or less minimum dates, but our opinion is that the present date is fairly reliable. It fits fairly well with the chronology developed by de Vries (*3*, *4*).

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4. We thank J. van Wel for the construction of the columns. The analyses of O^{18} were carried out by the mass spectrometric group of the Laboratory for Mass Spectrography in Amsterdam. The isotopic enrichments were carried out as part of the research program of the Stichting voor Fundamenteel Onderzoek der Materie with the support of the Nederlandse Organisatie voor Zuiver Wetenschappelijk Onderzoek.

28 March 1958

Extending the Range of Dose-Effect Curves for Irradiated Mice

A recurring problem in experiments yielding results to be expressed in the form of dose-effect curves is the need to use responses measured in different units at different parts of the dosage scale. Any attempt to combine two such responses rests on the assumption that they reflect the same phenomena. A number of methods for extending the dosage scale by combining percentage survival and survival time into a single response have been proposed (*1*). We report here a simple method for combining information on survival which we have recently found useful in our studies on the effect of colchicine and its derivatives in irradiated mice (*2*).

When given 24 hours before exposure to radiation which would kill all controls in an average of 9 days or longer, trimethylcolchicinic acid methyl ether d-tartrate increased the percentage surviving. When given to animals which radiation would kill in 8 days or less, no effect on survival was obtained. After the results were plotted in the usual way (upper half of Fig. 1*A*), the ordinate at zero survival was equated with the mean survival time of the animals which had received the lowest radiation dose required for approximately zero survival (in these experiments, 1000 r and 10.5 ± 0.6 days). At higher radiation doses, survival time gradually decreases to a plateau value of approximately 4 days (*3*). It was noted that this plateau was reached at a radiation dose which is the same distance above the approximate LD_{100} as the dose which would kill almost no animals is below the LD_{100} (1300 r, 1000 r, and 700 r). The ordinate was extended downward to 4 days, and the distance between 4 and 10.5 days was made to equal the distance between 0 and 100 percent survival, with the LD_{100} becoming, on the combined scale, the mid-dose, which for a single response would be the ED_{50} (lower half, Fig. 1*A*). The data, when plotted in this way, yield a smooth radiation dose-effect curve, permitting an expression of percentage mortality and mean survival time

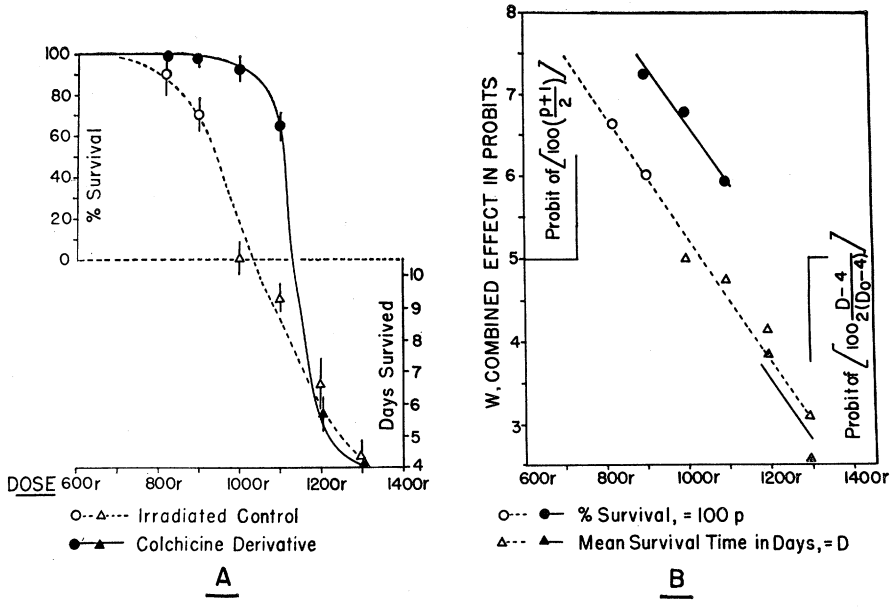

Fig. 1. (*A*) Plot of percentage survival and mean survival time by radiation dose for control mice and those treated with a colchicine derivative (1 mg of trimethylcolchicinic acid methyl ether *d*-tartrate, administered intraperitoneally 24 hours before irradiation). (*B*) Same data plotted in probits.

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.

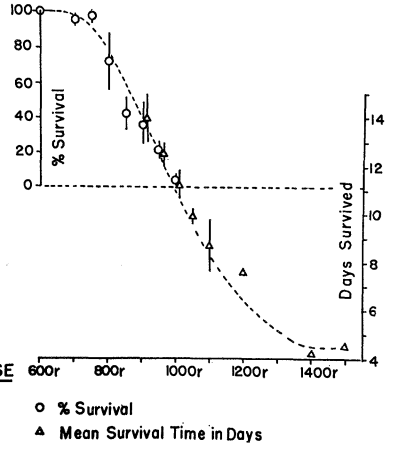

Fig. 2. Plot of percentage survival (open circles) and mean survival time (open triangles) by radiation dose for 66 control groups of mice not represented in Fig. 1.

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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