days at 25°C, the cell crops were collected. The cells were first extracted with methanol and then with freshly distilled ethyl ether free of peroxides. The extracts were mixed up and concentrated under reduced pressure. The residual extract was saponified in 10 percent alcoholic potassium hydroxide solution at room temperature for 10 minutes. The solution was diluted with water and extracted with ethyl ether. The ether extracts were evaporated to dryness in a vacuum.

The residual mass thus obtained was dissolved in a small amount of petroleum ether (boiling point, 30° to 50°C) and allowed to flow onto columns which consisted of layers of calcium hydroxide and calcium carbonate. By chromatographic separation, only the original strain was ascertained spectrophotometrically to contain δ-carotene and rubixanthine (*1*). The optical densities of the extracts, at wavelengths from 370 to 530 mμ, are shown in Fig. 1.

Each strain highly resistant to tetracycline was colorless, without exception. The lack of color is not attributable to lipoxidase, for it was ascertained, by the linoleic acid method (*2*), that not only the sensitive strain but also the resistant ones were free of lipoxidase activity.

As is shown in Fig. 1, a strain highly resistant to oxytetracycline has no absorption from 370 to 530 mμ after treatment by the same method for extraction of carotenoids as was employed with the sensitive strain.

From these experimental results, it may be suggested that tetracyclines block a step or steps on the pathway of biosynthesis of carotenoids.

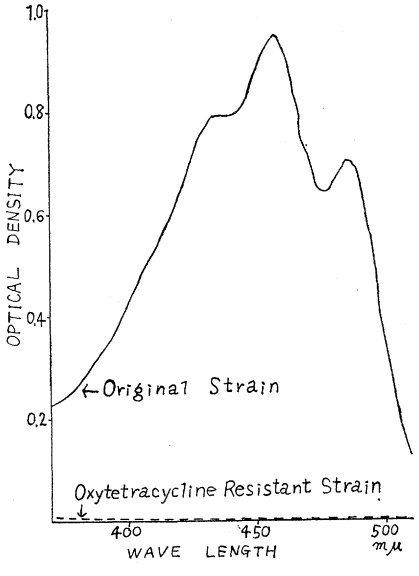

Fig. 1. Optical density versus wavelength for extracts of carotenoids from *Micrococcus pyogenes* var. *aureus* 209 P. (Solid line) Original strain; (dashed line) oxytetracycline (300 μg/ml) resistant strain. Solvent, *n*-hexane; concentration of extract, that obtained from 20 mg (dry weight) of cells per milliliter of *n*-hexane; instrument used, Beckman spectrophotometer model DU.

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Half-Life of Sulfur-35

Abstract. A new determination has been made of the half-life of the beta emitter sulfur-35. Approximately 400 measurements were taken over a period of a year and a half. These data were corrected for the dead time of the counter and then treated statistically. The half-life was found to be 86.35 ± 0.17 days.

One of the most commonly used radioisotopes in chemical and biological tracer experiments is S^{35}. For accurate work, it is necessary to make a correction for the decay of the isotope; this requires a precise knowledge of the decay rate. The uncertainty associated with the presently accepted half-life of S^{35} limits the accuracy of certain types of experiments. Accordingly, we undertook to determine a more precise value for the half-life.

The decay rate λ is defined by the equation

$$\ln N - \ln N_0 = -\lambda t \qquad (1)$$

where N_0 is the initial count rate and N is the count rate at time t. It is clear from this equation that if only one radioisotope is present, ln N will be a linear function of time. Thus, radioactive contamination of a radioisotope can be detected by a nonlinearity in this relationship. A secondary objective of our experiment was to determine whether such contamination was present.

The S^{35} sample was in the form of $CaS^{35}O_4$ deposited on a copper planchet. A thin layer of clear Krylon was placed over the source to prevent the loss of radioactive material.

The planchet containing the source was placed in one of the wells of a shielded, gas flow counter. A C^{14} source consisting of a thin plastic film mounted in a planchet was placed in the second well, and the third well was used for background measurements. The C^{14} was used as a constant source to check the efficiency of the counter and insure that it did not change over the period of the experiment. These sources were not touched during the entire experiment, so that each geometry remained the same. The well counter protected the sources from dust which might have absorbed part of the beta radiation, and a visual inspection before and after the experiment indicated that the appearance of the sources had not changed.

Counts were taken at a standard time each day for periods of 10 minutes each on the three wells of the flow counter. Four hundred and one sets of measurements were made over a period of 500 days. During this time the mean background rate was 24 count/min (range, 21 to 27 count/min), and the C^{14} readings were constant within 1 percent. The initial counting rate of the S^{35} was approximately 1300 times the background rate; by the end of the experiment about 1½ years later, the counting rate had decreased to about 30 times background.

Because the counting rate was fairly high, a correction had to be made for the counts lost during the dead time of the counter. A measurement of the resolution was made by the standard method of splitting a planchet into two pieces and placing a drop containing the S^{35} compound on each. The counting rate was then measured for each drop separately and for the two together. The dead time is given by

$$\tau = \frac{2(n_1 + n_2 - n_3)}{(n_1 + n_2)n_3} \qquad (2)$$

where n_1 and n_2 are the counts due to the separate drops and n_3 is the count when both drops are measured together. The dead time found for the flow counter used in this experiment was 149.1 μsec, which agrees well with the manufacturer's specifications.

Because the variation in the C^{14} counts was small, no correction was made for detector efficiency. The background count measured each day was subtracted from the S^{35} count, and the difference was taken as the measured count for that day. In order to obtain the actual count, a correction was made for the counts lost because of the finite dead time of the counter. The measured count can be written as

$$n = N - nN\tau$$

where N is the actual count and τ is the dead time of the counter (Eq. 2). Since n and τ were known, a value for the actual count, N, was found for each measurement.

$$N = n/(1 - n\tau) \qquad (3)$$

During the early part of the experiment when the counting rate was high, the

correction for dead time was about 9 percent. This fell off to only a fraction of a percent correction at the end.

The natural logarithm of N varies linearly with the number of days. In order to find the best-fitting straight line, a regression coefficient of ln N upon the time t was calculated. The regression formula can be written (*1*)

$$\underline{\text{Ln } N} = \overline{\ln N} + b(t - \bar{t}) \qquad (4)$$

where $\underline{\text{Ln } N}$ is the predicted value of ln N; t is the time in days from the starting point; $\bar{t}$ is the mean of $t_1, t_2, \ldots t_{401}$; $\overline{\ln N}$ is the mean of ln N_1, ln N_2, . . . ln N_{401}; and b is the regression coefficient, which for this case is

$$b = \frac{\Sigma t \ln N - 401\,\bar{t}\,\overline{\ln N}}{\Sigma t^2 - 401\,\bar{t}^2} \qquad (5)$$

By applying this equation to the data, a value was found for b. By comparing Eq. 4 with Eq. 1, it is seen that the regression coefficient b is the negative of the decay constant, λ. Thus the half-life can be found by substituting $-b$ in the well known equation

$$t_{1/2} = (\ln 2)/\lambda \qquad (6)$$

The regression is the line which on an average gives the minimum standard error. To determine the degree of linearity of the relationship between time and ln N, it is necessary to calculate the correlation coefficient. This is defined as the square root of the ratio of the sum of squares due to regression over the total sum of squares. If this coefficient is 1 or -1, the total variation is then due to the regression and the relationship between the variables is perfectly linear. Any contamination of the S^{35} source by other radioactive material would be indicated by a deviation of the correlation coefficient from an absolute value of 1.

In order to estimate the limits of error of the half-life, the standard error of the slope of the regression line was calculated. By adding this standard error to, or subtracting it from, the slope, its effect on the half-life was determined.

The presently accepted half-life of S^{35} is 87.1 ± 1.2 days. This value was found by Hendricks *et al.* (*2*) by least-square fit of 189 points. The correlation coefficient of the best-fit curve for this work was 0.969 and the standard deviation of the count data from the curve was 6 percent. A very weak source was used, resulting in a maximum count which was only 3.1 count/sec above background and a minimum of only 0.7 count/sec above background. Earlier work by Levi (*3*) indicated a value for this half-life of 88 ± 5 days. This value, however, is based on less than 40 points taken over a period of 500 days.

The value for the half-life of S^{35} determined in the present experiment is 86.35 ± 0.17 days. The correlation coefficient was found to be $-.9993$. This value, being very close to -1, indicates a high degree of linearity and disposes of any possibility that the source contained radioactive material other than S^{35}. The half-life measured here was nearly 0.8 day less than that found by earlier investigators but was still within their calculated error. The uncertainty in this measurement is considerably smaller than that of Hendricks *et al.* both because of the higher counting rate used and because of the fact that more than twice as many points were taken.

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Geographical Pattern of Crotamine Distribution in the Same Rattlesnake Subspecies

Qualitative individual differences in the composition of the venom of the same ophidian species are of fundamental importance in snake-bite pathology and therapeutics, since, as a rule, ophiotoxicosis results from the venom of a single snake. Knowledge of venom variations and their geographical distribution leads to securing venoms with a more specific composition, and this will facilitate investigations of snake venom. The geographical distribution of these differences in composition would also throw some more light on the phylogeny and genetics of poisonous snakes. Investigations now in course in our laboratory show that qualitative differences in venom composition, within species or subspecies, are common, at least among Brazilian vipers.

In the present paper a particular example of venom differences in the South American rattlesnake [*Crotalus terrificus terrificus*) or, according to Klauber's revision, *C. durissus terrificus* (*1*)], is presented, and the geographical distribution of two biochemical variants in this subspecies, one with and the other without crotamine in its venom, is shown.

Moura Gonçalves (*2*) isolated, by electrophoresis, from the venom of the South American rattlesnake, a very toxic protein to which he gave the name of crotamine. He also observed that crotamine is present in the venom of some specimens of this subspecies, while its presence cannot be detected in the venom of other specimens (*3, 4*). This fact induced Moura Gonçalves to recognize in this form a biological subspecies which he denominated *Crotalus terrificus crotaminicus* (*3*). However, whether crotamine could alternately be present or absent in the venom of the same individual rattlesnake remained to be verified. Its absence would substantiate the existence of biological subspecies variants, while its constant presence in the venom of individual rattlesnakes would permit the study of geographical distribution of the two types (crotamine-positives and crotamine-negatives), adding further ground for recognizing such crotalic subspecies variants.

The paralysis in extension provoked by crotamine in hind legs of mice, as represented by the contraction of the leg extensor muscles, was used in this work to test the presence of crotamine in individual extractions from 530 rattlesnakes. An apparently high dose of venom (0.5 mg per mouse) was tentatively chosen for each test in order to detect crotamine in venoms where this toxin could be found in small concentrations. Two mice were employed for each assay, the venom being injected subcutaneously. It was found, later on, that in a few cases this dose was not sufficient to test venoms with a low crotamine concentration. However, because of the scarcity of venom in some extractions, the dose of 0.5 mg was used. With the venom of some rattlesnakes from northeastern Brazil (state of Ceará) having low crotamine concentration, only doses of 1 mg provoked positive responses. The venom of some rattlesnakes from southern Bra-

Table 1. Distribution and presence of crotamine in rattlesnakes.

Assays per snake (No.)	Snakes (No.)	Assays for group (No.)	Observation period (mo)	Crotamine	
				Positives (No.)	Negatives (No.)
1	431	431	—	252	179
2	5	10	1	3	2
3	15	45	2	10	5
4	30	120	3	16	14
5	10	50	3	5	5
6	4	24	4	1	3
7	4	28	5	1	3
8	3	24	6	3	0
9	7	63	7	3	4
10	5	50	8	4	1
11	4	44	9	3	1
12	1	12	9	0	1
13	1	13	10	1	0
14	1	14	10	1	0
18	9	162	14	7	2
—	530	1090	—	310	220