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

zil, considered as crotamine-negatives, might eventually show crotamine in their venom if the venom were assayed with larger doses, although many determinations made thus far on snakes from this region, with 1 mg of venom, have shown no differences from the results obtained with 0.5 mg.

Electrophoretic determinations, however, are more suitable than the pharmacological assays to detect crotamine in venoms where this toxin is found in low concentrations. On the basis of the sensitiveness of both methods, it is possible to state the existence of crotalic venoms exempt of crotamine. The reliability of the pharmacological assay used in this work is satisfactory, and the results reported by Moura Gonçalves (*3*), obtained with electrophoresis, are comparable to ours for the same localities. The peculiar geographical distribution corresponding to either type of our rattlesnake also contributes to corroborate both the pharmacological assay and the 0.5-mg dose. Small modifications that could be made on account of dose insufficiency would alter the results very little.

From the 530 rattlesnakes a group of 99 was separated. Several (2 to 14, Table 1) extractions from each snake were tested during the time it survived in captivity.

In this group, the individual secretion showed a constant composition, with regard to crotamine, in all extractions examined; the venom assay of these 99 rattlesnakes revealed 58 that were crotamine-positives, the remaining 41 being crotamine-negatives. The fact that some rattlesnakes always secrete crotamine and others never do show that this secretion may be related to a genetic character.

The geographical distribution of both crotamine-secreting and -nonsecreting rattlesnakes is shown in Fig. 1, where it can be seen that east of the 49th meridian and south of the 22nd parallel the crotamine-negative rattlesnakes (crossed circles) predominate, although, in this region, the two types cohabit in a mixed distribution so that, in the same locality, specimens with the two venom variants may be found. This fact may indicate that in this region the rattlesnakes are in a hybrid condition. To the west of the states of São Paulo and Paraná, an increase in the number of the crotamine-positive rattlesnakes (black circles) is observed until a region is reached where only crotamine-positive rattlesnakes are found (crotamine region). In the map, a dot-and-dash line separates the two regions. The rattlesnakes in the crotamine region, apparently are still in a genetically pure condition. The two rattlesnake variants seem to be distributed in geographical mosaics, since in very distant places, beyond those shown on the map, is found either one or the other type of rattlesnake, or both together; thus, we detected crotamine in the rattlesnake venom from the state of Ceará (northeastern Brazil); other workers (*2–5*) have verified that in pools of rattlesnake venom from the state of Goiás (middlewestern Brazil), in northern Brazil, and some places of northeastern Brazil, only the noncrotamine-secreting rattlesnakes can be found. The rattlesnakes of Argentina (*5*) all appear to be crotamine-positive.

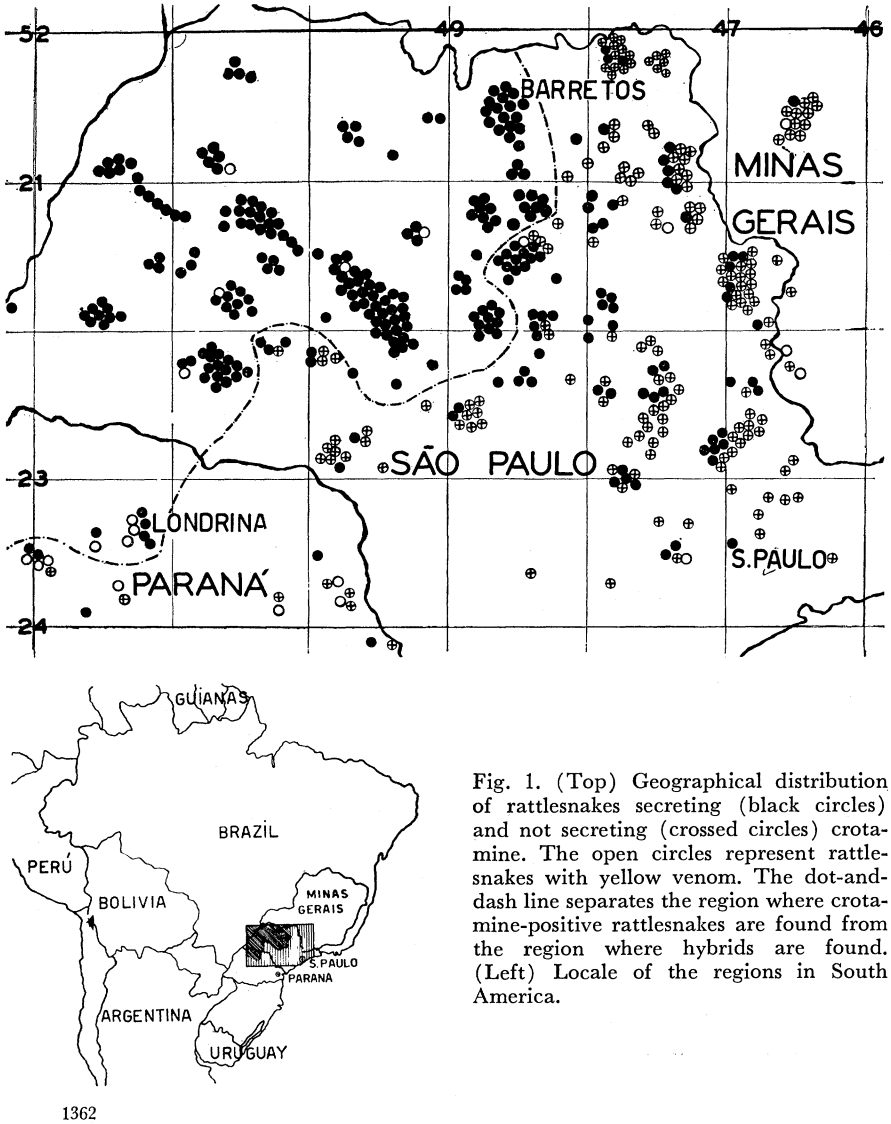

Fig. 1. (Top) Geographical distribution of rattlesnakes secreting (black circles) and not secreting (crossed circles) crotamine. The open circles represent rattlesnakes with yellow venom. The dot-and-dash line separates the region where crotamine-positive rattlesnakes are found from the region where hybrids are found. (Left) Locale of the regions in South America.

In regard to color, the dried crotalic venom can be either white or yellow, the yellow color being related to its content of ophio-L-amino acid oxidase. South of the 23rd parallel rattlesnakes having yellow venom predominate (open circles). The crotamine region (white venom), extends from western São Paulo into northern and northwestern Paraná. In the latter state a small area is discriminated, in the lower part of the crotamine region, where the rattlesnake venoms contain crotamine and are yellow in color; here, a new component (yellow component) is superimposed on the crotaminic venom which is responsible for the change in the venom color.

These investigations show the following. (i) The venom gland of rattlesnakes seems to be genetically provided to secrete or not to secrete crotamine. (ii) Two geographical regions can be delimited with regard to crotamine secretion, one where only crotamine-secreting rattlesnakes are found (genetically pure), the other (larger) where crotamine and noncrotamine-secreting rattlesnakes are found in mixed distribution (hybrid region). This geographical distribution also appears to confirm the Mendelian character of crotamine secretion. (iii) More data are added to the discrimination of the two rattlesnake variants which induced Moura Gonçalves to recognize *Crotalus terrificus crotaminicus* as a "biological" subspecies. (iv) Investigations in many directions might be helped if morphological data could be checked by venom composition criteria in poisonous snake taxonomy. (v) The generally accepted concept of venom composition homogeneity in snake species must be re-

garded with caution, since investigations of venom composition are usually made with pools of extractions from a large number of snakes, and the individual venom composition is thus hidden in the species' venom pool (*6*).

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High-Energy Phosphates during Long-Term Hibernation

Abstract. Adenosine triphosphate (ATP) and phosphocreatine (PC) show contrasting levels in muscle and liver after short and long periods of hibernation. In prolonged hibernation cardiac and skeletal muscle PC continues to maintain ATP, but at lower levels. In the liver, control levels for these compounds are regained.

During the course of experiments with hibernating ground squirrels, *Citellus tridecemlineatus,* we were fortunate in having nine animals hibernate for a month without interruption. Since past reports (*1*) have pertained to an uninterrupted hibernation period of 3 to 5 days (short-term hibernation), we decided to compare those results with results obtained from the animals that hibernated a month (long-term hibernation).

The control animals were kept in an environmental temperature of 25° to 27°C and were anesthetized with Nembutal before sacrifice. The hibernating animals were kept in a cold room at 3° to 5°C, and tissue was removed for analysis within 30 seconds after the animal was first handled. At the time of sacrifice the thoracic cage was opened, and the heart was frozen *in situ* with mixture of ether and Dry Ice and removed. The same procedure was applied to the liver and the muscle from the hind limbs. For phosphate analysis 0.85- to 1.00-g samples of tissue were extracted with cold 10-percent trichloroacetic acid at 0°C, and the determinations were based on the method of Fiske and Subarrow (*2*). Inorganic phosphate (IP) was precipitated with calcium at an alkaline *p*H (*a*); inorganic phosphate, including phosphocreatine, was hydrolyzed with molybdic acid at room temperature for 30 minutes (*b*); and total acid-soluble phosphate was hydrolyzed by heating at 100°C in 1*N* HCl for 8 minutes (*c*). Accordingly, the following values are obtained: IP = *a*; PC = *b* − *a*; and APP = *c* − *b*. Adenosine polyphosphate (APP), which is actually a combination of adenosine triphosphate and adenosine diphosphate, is reported in this paper as adenosine triphosphate (ATP). Glycogen was determined by the procedure of Kemp and Van Heijningen (*3*).

The results show that when cardiac muscle of the long-term hibernators is compared either with that of the controls or that of the short-term hibernators, ATP ($P < .05$) and PC ($P < .01$) decrease significantly and to approximately the same degree (Fig. 1). Apparently the longer the heart beats at the extremely slow hibernating rate of 15 to 25 beats per minute, the smaller the high-energy phosphate content. Since the ATP/PC ratio is greatest (5/1) at this time, most of the high-energy phosphate present is ATP, the "active" form which is supplying energy, at the expense of PC, to the slow but continually beating heart.

Although skeletal muscle shows a significant ($P < .01$) decrease of 49 percent in both ATP and PC when long-term and short-term hibernation are compared, the ATP/PC ratio is the same, 1/1. The quantities are smaller, but when one takes into consideration the lack of movement over a longer period of time, this is plausible.

In the liver ATP is decreased 55 percent ($P < .01$) and PC is increased 107 percent ($P < .02$) when the long-term hibernators are compared with the short-term ones. However, when the short-term hibernators are compared with the controls the reverse is true; ATP is increased 120 percent ($P < .01$) and PC is decreased 62 percent ($P < .01$). It appears that the high-energy phosphate compounds of the long-term hibernators have reached an equilibrium comparable to that of the controls.

Glycogen values of the long-term hibernators, when compared with those of the short-term hibernators, showed the following decreases: cardiac muscle, 36 percent; skeletal muscle, 3 percent; liver, 9 percent. None of these changes are significant.

An increase in the hibernating period from 5 to 30 days decreases both ATP and PC in cardiac muscle. Phosphocreatine shows the greater decrease because it is maintaining ATP which is considered the "active" form (*4*). Although the high-energy phosphate content decreases in skeletal muscle, the ATP/PC ratio remains approximately the same. The slowly beating heart is using ATP and depleting PC while the stationary limb muscle maintains metabolic function at the 1/1 ratio of short-term hibernation with lower levels of the compounds. The increase in liver in organic phosphate during long-term hibernation is difficult to explain but may be due to an increased breakdown of organic phosphate compounds resulting from the interruption of the activity of certain enzyme systems. With metabolic transformations apparently at a minimum, ATP and PC have resumed control levels.

In the regulation of metabolism, gly-

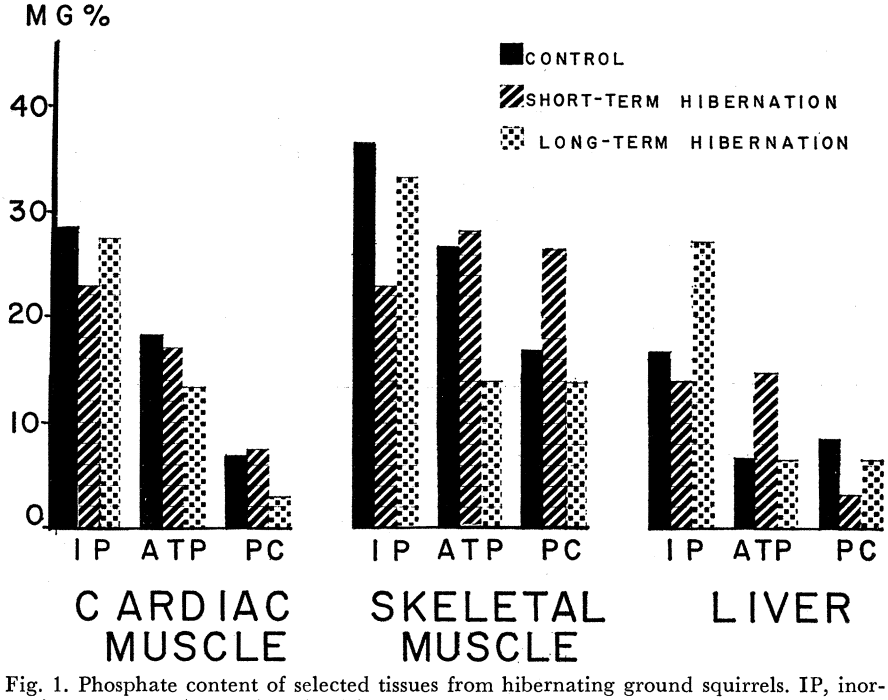

Fig. 1. Phosphate content of selected tissues from hibernating ground squirrels. IP, inorganic phosphate; ATP, adenosine triphosphate; PC, phosphocreatine.