was taken. The samples were dried in 1-cm copper planchets and counted in a gas flow counter. Corrections for self-absorption were not necessary on the diluted samples. On the others, a known amount of the labeled amino acid was added to occasional samples; these were then dried and counted, and the absorption factor was calculated. This value did not vary significantly within any one peak. That part of the effluent curve showing glycine and alanine is reproduced in Fig. 1, together with the calculated specific activities for the individual fractions. Qualitatively similar results were obtained with all the amino acids studied.

It is apparent that the presence of C^{14} in an amino acid molecule resulted in slower movement on the column. Consequently, there was a progressive increase in the specific activity of the fractions within a peak. In large part, the observed differences involved singly labeled molecules, for the amino acids were uniformly labeled only in a statistical sense, and the level of radioactivity in the source material used for their biosynthesis corresponded to a C^{14} to C^{12} ratio of approximately 1 to 10 (*5*). It follows that in the case of glycine, for example, approximately 18 percent of the total counts would have been contributed by doubly labeled compound.

If it is assumed that each pair of curves can be represented by the same distribution curve with different means, an equation relating specific activity to fraction number can be derived from the ratio of the two distribution curves (*7*). For a normal distribution, the logarithm of the specific activity proves to be directly proportional to the fraction number in the region where the peaks overlap. The slope constant describing this linear relationship is a measure of the degree of resolution of the labeled and unlabeled compounds. The slopes for the seven known amino acids here studied are given in Table 1. Also included are the slopes obtained from the chromatography of a hydrolysate of total protein from cells fed uniformly labeled glucose. It is apparent from Table 1 that there was an extremely close relationship between the ratio of C^{14} to total C in a labeled molecule and the degree of resolution. Using the slopes listed in Table 1 (the average when two figures were available) the correlation coefficient was in fact 0.96. The correlation coefficient between slope and molecular weight was −0.74. This suggests that the effect of a C^{14} atom on chromatographic behavior may depend on its position in the molecule, rather than solely on its mass. The larger the number of carbon atoms, the smaller would be the chance that a single C^{14} atom would appear in one of the more effective positions. This working hypothesis is supported by the observation that the specific activity slopes of glutamic acid and proline derived from glutamine-2-C^{14} were more than twice those observed for the randomly labeled compound.

Table 1. Slope of curve relating the logarithm of the specific activity of randomly labeled amino acids to fraction number. The slopes are given in arbitrary units; A, known amino acid mixture; B, protein hydrolysate of cells fed labeled glucose.

Amino acid	Ratio* of C^{14} to total C	Slope	
		A	B
Glycine	1/2	100	108
Serine	1/3	62	51
Alanine	1/3	59	66
Aspartic acid	1/4	57	54
Threonine	1/4	32	
Valine	1/5	24	
Proline	1/5	21	
Glutamic acid	1/5		16

* Ratio per labeled molecule, assuming a single label.

Isotope effects have been noted in a number of biological and chemical systems (*8*) as well as in behavior on ion-exchange chromatography (*9*). A slight concentration of natural isotopes by ion exchange was first reported for Li^6 and Li^7, K^{39} and K^{41}, and N^{14} and N^{15} by Taylor and Urey (*10*) and by Brewer (*11*), who used 35-ft and 100-ft columns of a natural zeolite. Recently, a 780-fold concentration of N^{15}-labeled ammonia to 74 mole percent N^{15} has been effected by repeated cycling through 5-ft columns of Dowex 50 for a total of 600 ft (*12*). Tritrium-labeled organic acids have shown a similar isotope effect on partition chromatograms (*13*). In contrast, the same technique failed to change the isotope ratio of C^{14}-labeled formic acid significantly. The difference in chromatographic behavior between the labeled and unlabeled amino acids in the present experiments was small; but it is possible that with much larger columns and repeated cycling, an isotopic enrichment could be developed similar to that accomplished for $N^{15}H_3$ (*12*).

Coincidence of radioactivity and ninhydrin color has been widely used as a criterion of identity in the study of labeled amino acids and related compounds. This seems to be valid for paper chromatography, but it is not valid for the newer methods of ion exchange chromatography with their extremely high resolving power. However, a straight line relationship, with the correct slope, between the logarithm of the specific activity and the fraction number would serve as a criterion of homogeneity. There is a large possible error if a single fraction, rather than the entire peak, is used for the determination of specific activity.

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7. Thus,

$$\ln S = \left(\frac{m_1 - m_2}{\sigma^2}\right) x + \frac{m_2^2 - m_1^2}{2\sigma^2}$$

where S is the specific activity, m_1 is the mean of the activity curve, m_2 is the mean of the ninhydrin color curve, σ is the standard deviation, and x is the fraction number.
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28 September 1955

Thallium Acetate in the Diagnosis of Chronic Respiratory Disease of Chickens

Thallium acetate (TA) was first recommended in 1947 as a bacteriostatic agent in the isolation of pleuropneumonialike organisms (PPLO) in liquid medium and on plate cultures (*1*). In primary isolations where contamination was usual, TA in proper concentration inhibited ordinary bacteria and allowed PPLO to grow in pure culture. In another study of PPLO of human origin (*2*), TA used as a bacterial inhibitor in liquid and solid media did not produce "L" forms from bacteria, possessed a wide bacterial spectrum, inhibited both gram-positive and gram-negative organisms, and had a selective action independent of the total protein content of the culture medium.

Chronic respiratory disease (CRD) of chickens, caused by a PPLO (*3*), is diagnosed by isolation of the agent in em-

bryonating chicken eggs or on artificial medium. In a study of the etiology and pathology of the CRD complex of chickens (*4*), the agent was isolated by the following procedure. Tracheal scrapings from a chicken were added to broth and penicillin was added to control bacterial contamination. Penicillin does not affect PPLO (*5*) but does suppress growth of certain contaminating bacteria. After this mixture had been incubated, a portion was injected into the yolk sac of embryonating chicken eggs. The presence of the CRD agent was revealed by specific respiratory lesions in the embryo.

In a later investigation of field cases (*6*), contaminated suspensions were retested. However, in addition to penicillin, 0.15 ml of aqueous 5-percent Seitz-filtered TA solution (*7*) was added to each milliliter of the broth suspension containing the tracheal scrapings. Treated suspensions were subjected to several serial embryo passages to determine whether the CRD agent was present. The harvested allantoic fluid from the serial passages was bacteriologically cultured, and TA was not added after the first embryo passage. Characteristic respiratory lesions in the embryo were regarded as positive evidence that the scrapings contained the CRD agent.

Use of TA made it possible to identify CRD in seven of ten cases in which the tracheal suspensions were heavily contaminated with bacteria. Unfortunately, TA may prove toxic for embryos. Some embryos inoculated with the suspension containing TA died early. Others exhibited marked stunting, underdevelopment of the mandible, and micromelia. When TA alone was inoculated into yolk sacs of 7-day-old embryos, the same teratologic syndrome affected most embryos (Fig. 1).

A similar syndrome has followed injection of insulin, boric acid, or pilocarpine hydrochloride into developing 4-day-old chicken embryos via the yolk sac. The best method to reduce or prevent this teratologic effect was to inject nicotinamide (NA) simultaneously. The protective effect of NA suggested that the skeletal defects were caused by a disturbance of carbohydrate utilization (*8*).

Another series of experiments was therefore carried out to determine whether NA would neutralize the effect of TA. The following mixtures of a 5-percent solution of NA and a 5-percent solution of TA were tested: (i) 0.5 ml TA and 0.5 ml NA; (ii) 0.3 TA and 2 ml NA; and (iii) 0.15 ml TA and 2 ml NA. After 10-min incubation, the material was inoculated into yolk sacs of 7-day-old embryos. NA did not neutralize the teratogenicity of TA. The concentrations of NA employed may not have been adequate, the embryos may have been too old, or the syndrome, although similar to the one described by Landauer (*8*), may have been caused by another type of disturbance in the developing embryo.

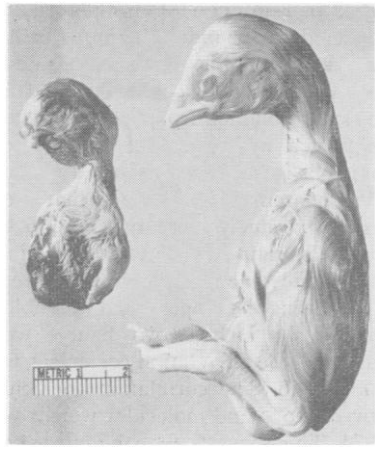

Fig. 1. Embryo on left: 14 days postinoculation. Yolk sac inoculated at 7 days with 0.2 ml of a dilute solution of thallium acetate (0.15 ml of a 5-percent solution in 1 ml of water). Stunting, micromelia, and shortened mandible. Embryo on right: Uninoculated control (same age as inoculated embryo). [Photo by C. Brandt]

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Isotopic Composition of Common Lead from Southern Africa

The apparent extreme age of some pre-Cambrian rocks in southern Africa has been further demonstrated by recent publication (*1*) of the isotopic composition of common lead minerals from this region, showing ages in excess of 2×10^9 years. This report (*2*) supplements the earlier work with isotopic analyses of 16 samples from pre-Cambrian rocks in southern Africa (Table 1). The experimental procedure used is described elsewhere (*3*).

The merits and limitations of the so-called "common lead age" method have been discussed by several writers (*4*). If the broad assumptions necessary for the method are granted, equations such as the following may be used for determining the time of mineralization, t_m (in billions of years), measured from the present:

$$x_m = 18.87 - 11.54\ (e^{.154\ t_{m-1}}) \quad (1)$$
$$y_m = 15.77 - 0.0836\ (e^{.972\ t_{m-1}}) \quad (2)$$
$$z_m = 39.15 - 42.74\ (e^{.0499\ t_{m-1}}) \quad (3)$$

x_m, y_m, and z_m are the ratios of Pb^{206}, Pb^{207}, and Pb^{208} to Pb^{204}, respectively, measured for each sample. The particular values of the lead constants shown were determined from a number of dated galenas at this laboratory (*3*). The three isotopic ages obtained from Eqs. 1–3 for each mineral are averaged together to give the age shown in Table 1. The error associated with each sample age is a measure of the spread in values of the three isotopic ages, compounded with a small uncertainty in the isotopic composition.

With the exception of the Elba (No. 5) galena, all the samples from the basement complex of Southern Rhodesia show great age, possibly indicating a single major period of mineralization in the range of 2.2 to 2.5×10^9 years ago. The time of the Elba mineralization is uncertain because of a considerable spread in the three isotopic ages (0.5 to 1.6×10^9 years). Nevertheless, the Elba sample probably represents a different and later period of mineralization.

Three samples (Nos. 8, 11, and 14) from South Africa give apparent ages that are considerably younger than their pre-Cambrian environment. The geologic environment for these three minerals makes a real age less than about 800 million years very unlikely. The isotopic and geologic data therefore suggest that these three leads may have been originally deposited with an anomalously large amount of radiogenic lead.

The Keimos (No. 15) galena contains excessive amounts of radiogenic lead, exhibiting an anomalous composition very similar to that characteristic of lead deposits in the Mississippi Valley (*3*, *5*). The composition of the Witwatersrand (No. 16) sample shows contamination with uranium lead and is similar to that reported by the Toronto group (*1*). No reliable age assignments can be made for either of these leads. In any event, the Keimos anomaly merits further investigation in view of an apparently normal geologic environment and derivation.