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Origins of Chirality in Nature: A Reassessment of the Postulated Role of Bentonite

Abstract. *Bondy and Harrington have proposed that selective binding of* L *isomers of amino acids and* D *isomers of sugars to bentonite is the mechanism by which the chirality of molecules in living cells was originally established. Further experiments indicate that the observations of Bondy and Harrington are better explained in terms of the effects of the binding to bentonite of the products of radiochemical decomposition.*

Recently (*1*) Bondy and Harrington described experiments that they interpreted as showing preferential binding to bentonite of the L isomers of amino acids (thus suggesting a possible origin of chiral molecules in nature). We have not been able to confirm a preferential binding of the L isomers of amino acids, and experiments described here suggest that the radioactivity bound to bentonite is largely due to the binding of radiochemical decomposition products.

Tritiated compounds are usually supplied at 98 to 99 percent purity, but they may have rates of decomposition as high as 1 percent per month. The products of decomposition "stick" to any insoluble macromolecules. It has been suggested that measurements of bound radioactivity should be expressed as a percentage of the total counts available to make it clear when the observed binding is within the range of the concentration of impurities (*2*). Results are quoted here in this form as well as in the form of picomoles per 10 mg of bentonite for comparison with the data of Bondy and Harrington. From their data the percentage of available counts bound was calculated. In most instances, these values were 4 percent or less. The exception was the binding of L-[^{3}H]leucine, for which the percentage bound was 45 percent at a concentration of $10^{-8}M$. This was not in agreement with the published dissociation constant of $4.6 \times 10^{-6}M$, which implies that 50 percent binding was reached only at a concentration 460 times higher. Bondy (*3*) has said that 45 percent binding was not observed at $10^{-8}M$, and he thought the figure should have been one-tenth of this amount. It is probably worth noting that Bondy and Harrington used L-leucine at a specific activity of 58 Ci/mmole and D-leucine at 1 Ci/mmole because at higher specific activity tritiated compounds are less stable (*2*).

The ^{3}H-labeled amino acids that we used were obtained from the Radiochemical Centre, Amersham. These were L-aspartic acid (15 Ci/mmole), D-aspartic acid (18 Ci/mmole), L-leucine (55 Ci/mmole), and DL-leucine (42 Ci/mole). Unfortunately, D-[^{3}H]leucine was no longer available. The use of DL-leucine meant that, if D-leucine were not bound at all, the counts bound with DL-leucine would be 50 percent of those bound with L-leucine of the same concentration. In Bondy and Harrington's data, D-leucine was bound to 15.3 percent the extent of L-leucine. With DL-leucine, an equivalent result would require the binding to be 57.7 percent of that of L-leucine. Unlabeled amino acids were obtained from Sigma Chemical Company.

Three samples of bentonite were obtained by courtesy of Volclay from their sites at Lovell, Upton, and Belle Fourch. One sample of Queensland bentonite was supplied by Minerals Ltd. (Australia). These samples were washed with hot alkali as described by Bondy and Harrington (*1*). In the washing process gritty contaminants were removed, and by centrifugation coarser and finer particles of bentonite were separated. The finer particles had a swollen diameter of 1 to 1.5 μm. The binding of counts by fine and coarse particles of bentonite was similar, an indication that the phenomenon is related to the mass or volume of the swollen particles rather than to the surface area.

Bentonite suspensions were dispersed by volume with a reproducibility of 3 percent in the counts bound to 10 mg of bentonite. Despite the alkaline wash, we found it necessary to sterilize the bentonite and dispense it aseptically. Otherwise, microbial growth on bentonite resulted in preferential binding of the L-aspartic acid as compared with D-aspartic acid. Similarly, the dilute solutions of ^{3}H-labeled amino acids were freshly prepared with aseptic precautions because, when a diluted solution was stored at 2°C for a week, an increase in binding of L-aspartic acid (from 0.2 to 0.6 percent) was observed. To minimize the differences between our procedures and those of Bondy and Harrington, we used 50 m*M* tris-HCl buffer (*p*H 7) after we found that binding was similar in other salt solutions and only somewhat reduced in water.

Counting of the supernatants of the bentonite suspensions established that the percentage of counts bound was too

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Table 1. Radioactive binding to bentonite in solutions of [^{3}H]leucine and [^{3}H]aspartic acid. Bentonite samples were shaken with the amino acids in 50 mM tris-HCl buffer at 30°C for 15 minutes (leucine) or 90 minutes (aspartic acid). The supernatant obtained on the centrifugation of bentonite sample 1 was each time used to measure binding with sample 2. The bentonite was washed rapidly and counted as described in the text.

Tritiated amino acid	Bentonite sample	Concentration ($\times 10^{-7}M$)	Available counts (%)	Concentration (picomoles per 10 mg of bentonite)
L-Leucine	1	3.6	0.043	0.15
L-Leucine	2	3.3	0.03	0.10
DL-Leucine	1	3.1	0.045	0.14
DL-Leucine	2	3.3	0.025	0.08
L-Aspartic acid	1	6.6	0.21	1.2
L-Aspartic acid	2	4.6	0.18	0.87
D-Aspartic acid	1	6.6	0.26	1.7
D-Aspartic acid	2	4.6	0.17	0.82

small to be measured in terms of a change in the supernatant solution. We measured the binding of radioactivity by counting the washed bentonite. The washing time was reduced as far as possible to minimize the reverse process. We were able to complete three washes in ice-cold buffer in 12 minutes by braking the centrifuge rapidly after each 1-minute centrifugation. The adequacy of this procedure was established by the fact that the third wash liquid had a count approximating that of the normal background. The samples of washed bentonite were counted in the same way as in the earlier study (*1*).

Using the procedures outlined above, we found that the binding to bentonite with $10^{-8}M$ amino acids was too low for accurate measurement. The amino acids were therefore used at concentrations of 2×10^{-7} to $6 \times 10^{-7}M$, and bentonite was used at 5 to 10 mg/cm^3 instead of 2 mg/cm^3. The maximum binding of [^{3}H]leucine was reached in 15 minutes as described in (*1*), but the maximum binding of [^{3}H]aspartic acid required an exposure of 90 minutes at 30°C.

The radioactive supernatants from the bentonite were recovered, and fresh samples of bentonite were incubated for the appropriate time. If the counts bound the first time were due to the amino acids, the removal of a small percentage of the available counts should not have reduced the counts bound to the second sample of bentonite by more than this small percentage. Table 1 shows data for the binding of leucine and aspartic acid to two successive samples of bentonite. With aspartic acid the second concentration was lower.

The concentrations used in these experiments were approximately 20 times those used by Bondy and Harrington (*1*). The levels of binding are nevertheless lower than the 26.8 and 4.1 pmole per 10 mg for L- and D-leucine (*1*). No preferential binding of the L isomer is apparent in our data. The higher binding of D-aspartic acid to the first sample of bentonite is not observed with the second sample; this result suggests a higher degree of contamination of the tritiated D amino acid. The decline in the leucine bound to the second sample of bentonite confirms the binding of material that is not leucine, since for a decline in 0.04 percent of the counts there has been a decline in bound counts of about 45 percent. This was further confirmed by the observation that bentonite bound more counts when [^{3}H]leucine had been heated. After 50 minutes at 100°C, the percentage of counts bound increased from 0.02 percent of a freshly prepared solution to 0.11 percent for $3.3 \times 10^{-7}M$ leucine. In all the experiments that we carried out the percentages of counts bound were within the known limits of impurity.

Bondy and Harrington (*1*) showed a reduction in the binding of ^{3}H-labeled L amino acids in the presence of excess unlabeled L but not D amino acids. We did not observe this result in our study where the addition of $10^{-2}M$ unlabeled D- or L-leucine to $3 \times 10^{-7}M$ labeled DL-leucine only reduced the binding from 0.07 to 0.06 percent with added L-leucine and to 0.05 percent with added D-leucine. Bentonite labeled with [^{3}H]leucine was washed and suspended in buffer or in buffer with $10^{-2}M$ of the L or D isomer. The same release of counts was observed under the three conditions.

Similarly, the addition of $10^{-2}M$ of either D or L unlabeled aspartic acid reduced the binding of $4.6 \times 10^{-7}M$ L-[^{3}H]aspartic acid from 0.14 to 0.02 percent of the available counts.

Thus no selective binding of L amino acids by samples of sterile bentonite from four sources was observed. The observed binding is largely attributed to the products of radiochemical decomposition because (i) storage and heating of the dilute solutions increased the percentage of counts bound, (ii) the counts bound were always below the known levels of impurity of the radiochemicals, and (iii) a second addition of bentonite bound 45 percent less leucine after a loss of only 0.04 percent of the total counts.

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Cultivation in vitro of the Vivax-Type Malaria Parasite *Plasmodium cynomolgi*

Abstract. *The vivax-type simian malaria parasite* Plasmodium cynomologi *was cultured in vitro by both the candle jar method and the continuous flow technique, with rhesus monkey erythrocytes and RPMI 1640 medium supplemented with Hepes buffer and human serum. After 6 weeks in culture, the growth of the parasite had permitted a* 5×10^6 *cumulative dilution of the original inoculum. Cultured parasites remained infective to rhesus monkeys and exhibited a reversible decrease in the ameboid behavior of their trophozoites.*

Plasmodium vivax contributes substantially to the negative socioeconomic impact of malaria in the developing world (*1*). This human malaria parasite is widely distributed and causes a recurrent and debilitating disease. *Plasmodium cynomolgi*, the simian counterpart of *P. vivax*, is often used in research directed at *P. vivax*. We now report the successful continuous cultivation of *P. cynomolgi*, the first vivax-type malaria parasite to become accessible to long-term studies in vitro.

Plasmodium cynomolgi occurs in Old

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