Amino acid content of atmospheric precipitation

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ABSTRACT

The examination of rainwater by paper chromatography for amino acid content over a period of several months has established the existence of a persistent and widespread phenomenon in which varying amounts of glycine, valine, alanine, glutamic acid, aspartic acid and leucine-isoleucine are detected.

In 1954, FONSELIUS of Sweden reported the occurrance of amino acids in rainwater and snow which he tentatively identified as glycine and alanine. In a related and sustained series of experiments, MUNCZAK (1960) working in Vienna, entrained air through very dilute acetic acid and detected variable amounts of amino acids. The data presented here confirm and extend FONSELIUS's (1954) original observations by providing quantitative data over an extended period of time on both free amino acids and those appearing after the hydrolysis of rainwater residues (including snow and hail). It would seem clear from these considerations that atmospheric amino acids are a widespread phenomenon.

Experimental

Rain samples, including snow and hail, were collected in a glass measuring cylinder by means of a funnel with a collecting area of 80 cm^2. This and all other glassware were rigourously cleaned in chromic acid each time before use and care was taken to avoid fingering the internal surfaces. After twice filtering through Whatman No. 1 filter paper, samples were evaporated to dryness. Residues for hydrolysis were redissolved in 6 ml of redistilled constant boiling hydrochloric acid and refluxed for 18 hours. 6 ml of the acid alone was used as a control. Residues were again obtained by evaporation. The residues, hydrolysed and unhydrolysed, were then redissolved in 0.25 ml of deionized treated distilled water and 100 μl aliquots together with standard amino acid solution subjected to unidimensional paper chromatography using a modified form of the system developed by HANES *et al.* (1961) in which no desalting is required. The amino acids were estimated on the paper after treatment with ninhydrin reagent. Leucine and isoleucine are insufficiently resolved to permit individual characterisation.

All results given are net results following correction, where necessary, by the appropriate control. The author feels it prudent also to record the fact that he is cognizant of the work by HAMILTON (1965) in which it is claimed that a single wet thumb print on glass, on subsequent hydrolysis, yields an amino acid pattern.

Eight 100 ml samples of London tap water were taken at intervals from March to July 1964 and treated as for rainwater samples, but were found to contain no free amino acids. All the distilled water used in these experiments had in addition been treated with an Elgastat deionizing unit: no amino acids were detected in this water either free or after the hydrolysis procedure had been carried out on 100 ml samples.

At a later date further 100 ml samples of London tap water were taken and subjected to the hydrolysis procedure. These results are shown in Table 1. For further comment on these results see discussion.

An important aspect of the analytical procedure is the necessity of distilling the constant boiling point hydrochloric acid used in the hydrolysis since it was found that even "Analar" acid alone could give a substantial amino acid pattern. This observation led to a most

TABLE 1. *mμ moles of amino acids from hydrolysed samples of London tap water and distilled/Elgastat-treated water taken at intervals in 1964.*

	2nd. Sep.	5th. Oct.		20th. Oct.		22nd. Dec.
	Tap water	Tap water (both Seitz filtered)	Distilled water	Tap water	Distilled water	Tap water
Leu/isoleu	16.8	4.58	—	4.58	—	6.33
Val	—	12.8	—	6.84	—	8.55
Gly	8.0	1.2	—	—	—	—
Ala	—	*T*	—	—	—	4.28
Glu	—	3.4	—	—	—	—
Asp	—	2.26	—	—	—	—

T = trace.

interesting subsidiary find which although it may have a bearing on the subject as a whole, is discussed more fully in the appendix.

Results

From Table 2 it is evident that glycine is both the most persistent and the most abundant free amino acid occurring in rainwater with valine second in importance, followed by lesser amounts of alanine, glutamic acid, leucine-isoleucine and aspartic acid in that order. The same sequence is seen with the total amino acids following hydrolysis. In addition to this, in Fig. 1 a parallel trend is discernable between the amounts of free and total amino acid nit-

TABLE 2. *mμ mols of amino acids found in samples of atmospheric precipitation collected in Central London over an area of 80 cm².*

	1964													
	June		July		Aug.		Sep.		Oct.		Nov.		Dec.	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Gly	30.0	No record	9.4	83.5	4.0	42.7	Nil		5.4	17.3	8.0	36.0	4.0	44.0
Val	*T*	available	6.0	67.5	—	40.2	rainfall		6.0	6.8	*T*	1.7	11.1	19.7
Ala	12.3		—	52.9	—	34.8			—	5.6	—	5.6	—	12.0
Glu	*T*		—	17.0	—	17.0			—	6.1	*T*	10.4	—	13.6
Leu/isoleu	—		—	42.0	—	39.6			—	0.8	—	3.8	—	9.2
Asp	—		—	12.8	—	37.2			—	4.5	*T*	6.0	—	5.3

1965								Total mμ each amino acid	
Jan.		Feb.		Mar.		Apr.			
A	B	A	B	A	B	A	B	A	B
18.7	50.6	5.4	29.3	8.0	81.5	10.0	69.0	102.9	453.9
22.2	30.8	5.0	5.1	2.5	8.6	9.4	20.8	62.2	201.2
6.8	9.0	3.4	3.3	—	6.8	3.3	20.8	25.8	150.9
2.1	8.2	—	19.7	—	15.0	1.4	13.5	3.5	120.5
—	5.3	—	3.1	—	2.3	3.2	12.9	3.2	119.0
—	3.0	—	—	—	—	—	—	*T*	50.8

'A' columns represent free amino acids and 'B' columns, amino acids after hydrolysis. *T* = trace.

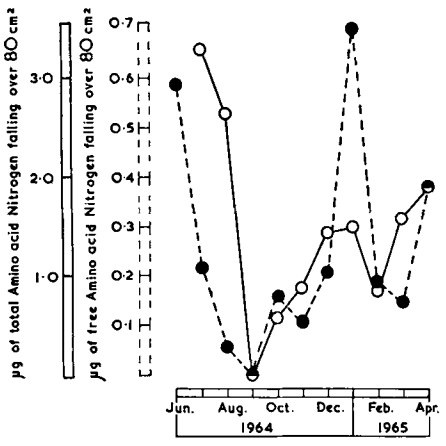

FIG. 1. μg of amino acid nitrogen brought down per month by atmospheric precipitation falling over an area of 80 cm² in Central London during 1964–1965.

rogen brought down per month in rainwater, and a tendency for the seasonal variation to display two maxima, one in Summer and another in Winter.

Although the bulk of the results obtained here relate to Central London, similar results have been obtained in other areas. Thus, simultaneous samples of rain collected in Central London and at Buckhurst Hill, some 12 miles north-east of Central London, within the period 12–15th June 1964, yielded comparable results as shown in Table 3. Evaluation of the valine-glycine ratio from Table 4 for rain collected in London compares favourably with that from Folkestone on the East Coast of England, with values of 1.8 and 1.6 respectively.

In an attempt to evaluate the size of particle or microorganism responsible for the amino acid content of rain, comparison of the total hydrolysed amino acid pattern of two identical samples of rainwater, one of which in addition to normal filtering had been filtered through a 0.9 micron Seitz filter, showed them to be identical. This puts an upper limit to the size of particle in the region of 0.9 microns. (The size in microns of a number of small bodies is given by TROMP (1963) as follows, condensation nuclei—0.01–50, pollen grains—10–180, viruses—(10–250) $\times 10^{-3}$, bacteria—0.2–100, algae—1–1000, spores—2–120.)

MUNCZAK (1960) in his air entrainement experiments used a filter of pore size as small as 0.25 microns and was yet still able to detect amino acids, though on some occasions there was a reduction in amounts.

In an experiment carried out in London similar to MUNCZAK's (1960), the hydrolysis of a sample of distilled water through which some 4.5×10^3 litres of air had been drawn in $2\frac{1}{2}$ days in early September 1964, yielded 0.2 μg of glycine and 0.5 μg of glutamic acid. A distilled water control through which no air had been passed was negative. Assuming the average base of a rain cloud to be at about 4000 feet (HUMPHREYS, 1943), the column of air above the rain collector, (80 cm² collecting area), to the base of the cloud might be expected to yield about 0.4 μg of glycine and 1.1 μg of glutamic acid. From the sample of rain collected nearest in time to this experiment, namely 18th–31st August 1964, the total amounts of glycine and glutamic acid found after hydrolysis were 1.25 μg and 1.0 μg respectively. In view of the uncertainties in the calculation, these two sets of results are closer than might be expected.

Discussion

It has long been established that the atmosphere is populated by a variety of microorganisms and related debris known collectively as the staubosphere (BLACKTIN, 1934) or aeroplankton (TROMP, 1963) and the scavenging of this material by rain provides per-

TABLE 3. *Results from samples collected simultaneously some 12 miles apart.*

	London	Buckhurst Hill
Volume of rain collected over 80 cm²	135 ml	105 ml
Total free glycine	0.9 μg	0.8 μg

together with similar amounts of alanine of the order of 1 μg and lesser amounts of glutamic acid and valine.

TABLE 4. *A comparative study of samples of rainwater from Folkestone on the East coast and Central London, collected within a few days of each other early in July 1964.*

	μg of free amino acids					
	Leu/isoleu.	Val	Ala.	Gly.	Glu.	Asp.
Folkestone (525 ml)	2.2	3.9	0.4	2.5	0.8	0.4
London (136 ml)	—	0.7	—	0.4	—	—

haps the simplest explanation for the amino acid content of rainwater.

The elevated values in Summer and Winter respectively for the curves displayed in Figure 1 bear comparison with those obtained by POLGAR & CSIKY (1964) in Hungary for the amounts of radioactive strontium brought down per month in rainwater during 1963. This would suggest a common scavenging effect of rain and that the actual maxima may simply be a reflection of the higher amounts of precipatation falling at the time. Clearly the results for September 1964 are an extreme example of this latter possibility when no rainfall was recorded in the collecting area. However, ERIKSSON (1952) gives results for the monthly amounts of ammonia nitrogen found in rain falling in Ottawa, Canada, where it is claimed that the rainfall varies very little from month to month and yet two maxima are evident, one in early Summer and another in Autumn. This would indicate that the amounts of scavengeable material are not solely dependent on the extent of the rainfall, other factors being at work.

An important point emerges from a comparison of the hydrolysed tap water results (Table 1) with the hydrolysed rainwater results (Table 2) when expressed as relative amounts of each totalized amino acid found in each system. Thus:

	Relative amounts of amino acid	
	Tap water	Rainwater
Leucine/isoleucine	14.2	2.3
Valine	12.5	4.0
Glycine	8.9	8.0
Alanine	1.9	3.1
Glutamic acid	1.5	2.4
Aspartic acid	1.0	1.0

From these results it is apparent that the rainwater pattern is markedly different from that of the tap water. This in turn clearly demonstrates that the technique used is differentiating between the two systems and invalidates any suggestions of systematic contamination occurring during the analytical procedure.

It is perhaps not entirely out of place to note that in tap water the leucine-isoleucine system is both the most abundant and persistent whereas in rainwater glycine is the most prominent amino acid with leucine-isoleucine as only a minor constituent. HILL (1963) quotes that the one amino acid that was found present in 50 % or more of twice weekly samples of seawater taken off the Scrips Institute of Oceanography pier over a period of 18 months was isoleucine.

The presence of free amino acids in rainwater might reasonably be supposed to arise from the partial hydrolysis of protein or peptide material. However, the scant amounts of glutamic acid, leucine-isoleucine and especially aspartic acid that are found free, despite their comparable stability with the other amino acids (BREGER, 1963), fall short of what might be expected from the post hydrolysis results. One possible explanation of this is that they are preferentially degraded by microorganisms soon after their release. Another explanation which if proved to be true would be quite interesting is that the free amino acids are not exclusively the result of partial hydrolysis but arise independently from some other source.

The data so far available do not permit a conclusive dismissal of the possibility of abiogenic processes as a cause of some of the amino acid content of rainwater. Thus the simultaneous samples of rain referred to in Table 3 were collected during an extensive thunderstorm prominent over both areas for some hours and the amounts of free alanine were by far the largest

recorded in these experiments. The possibility therefore of synthesis through the agency of lightning discharges presents itself. However, the more turbulent air movements and greater cloud height associated with storms may equally well be a contributory factor to the enhanced alanine values. No optical activity measurements have been attempted on the amino acids in this series of experiments principally due to their low concentrations in rainwater, though clearly such a study would be valuable in deciding the genesis of these compounds. No detailed attempt has been made regarding correlation in general with meteorological factors.

Although conditions in the lower atmosphere are essentially oxidative, both FONSELIUS (1954) and MUNCZAK (1960) consider the possibility of abiogenic processes, referring to the MILLER (1955) synthesis of amino acids from a mixture of gases subjected to electric discharges.

The phenomenon of atmospheric amino acids is most probably a composite one and more than one source would have to be sought to provide a total explanation. The presence of amino acids and other nitrogenous compounds have been reported in meteorites (HAYATSU, 1964), but their significance is a subject of dispute and the extent to which meteor dust might contribute to the amino acid content of atmospheric precipitation has not been computed. The ubiquity of sea spray (ODDIE, 1957) as a possible source for the amino acid content of the air might also be considered.

BALCARZYK & LANZIEZ (1965) have recently demonstrated the presence of viable carbonic anhydrase in the air, the concentration of which would appear to depend on weather conditions. Attention might be focused here on the post-hydrolysis amino acids of rainwater.

Based on the results given here, the total amount of amino acid nitrogen brought down annually in Central London is calculated to be 0.023 Kg N per hectare, and as might be expected, is less than the total albuminoid nitrogen (ERIKSSON, 1952). Whether this amount of amino acid material has any significance in relation to other systems, such as agriculture and human health has yet to be evaluated. Certainly some allergic conditions are traceable to specific material borne by the atmosphere; hay fever for example is excited by pollen grains (FLOREY 1958). In general, however, the subdivision of atmospheric albuminoid nitrogen into an amino acid component may provide a convenient parameter for wider studies.

APPENDIX

Some observations on the presence of amino acids in hydrochloric acid

The results of analysis by paper chromatography of 6 ml of a 1:1 mixture of "Analar" hydrochloric acid and deionized treated distilled water after refluxing for 15 hours were as follows:

Glycine	2.5 μg
Leucine/isoleucine	0.74 μg
Glutamic acid	0.67 μg
Alainine	0.25 μg
Aspartic acid	trace.

This and similar results led to the necessity of distilling the constant boiling point mixture of hydrochloric acid before using it for the hydrolysis of rainwater residues.

However, despite precautions to exclude contamination by atmospheric dust, such as storage of the distillate in a glass stoppered flask under an inverted beaker, the preliminary cleansing round the rim and stopper with fresh paper tissues and the rejection of a few mls. of acid before use in order to flush the neck of the flask, the amino acid pattern in the blank was observed to return after a week or more and apparently to increase with the age of the distillate. As a consequence of this, fresh preparations of redistilled acid became obligatory.

Eventually it was decided to examine this phenomenon in more detail. A quantity of freshly distilled acid was prepared and with the precautions previously indicated, aliquots were taken for analysis over a period of several weeks. The results are shown in Fig. 2, in which the amounts of the principal amino acid, glycine, found after hydrolysis are plotted against the time of sampling.

The curve is clearly logarithmic of the form,

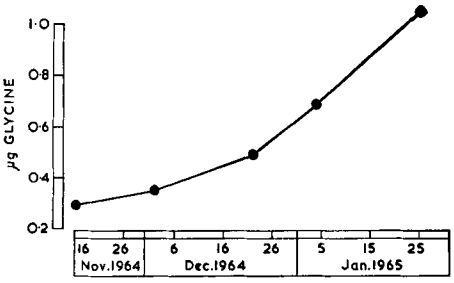

FIG. 2. μg of glycine found in successive 6 ml aliquots of initially distilled constant boiling hydrochloric acid kept in a glass stoppered flask in the laboratory.

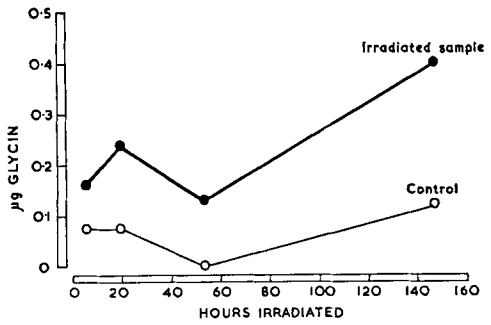

FIG. 4. μg of glycine found in successive 10 ml aliquots of initially distilled constant boiling hydrochloric acid subjected to prolonged U.V. light irradiation.

$y = a^t$, where y = amount of glycine, t = the time of sampling in days and a = a constant. Fig. 3 shows a plot of log. (μg of glycine × 10) against the time of sampling in days and is clearly a straight line through the origin.

Three alternative, major hypotheses can be considered.

(*a*) Ingress of foreign material from the surrounding air.
(*b*) A biogenic process as the result of an organism living in the acid.
(*c*) An abiogenic photochemical reaction involving the contents of the air and the acid.

(*a*) If contamination by atmospheric dust is offered as an explanation either by the ingress of ultra fine dust particles or the accumulative addition of material each time the flask is opened to take a sample, it would be difficult to reconcile the logarithmic form of the curve.

(*b*) The possibility of an organism replicating, let alone surviving in 5N hydrochloric acid seems improbable, and even more so in concentrated acid as in the case of the original observation in which the 1:1 mixture of acid—water was prepared from acid taken direct from the "Analar" reagent bottle. Nevertheless the remains of the distillate used to establish Figure 2 were examined for the presence of bacterial organisms by Dr. Townsend and Dr. Moss of the Institute of Tropical Products, London; results were entirely negative.

(*c*) Left with the interesting proposition that the phenomenon might be the consequence of a photochemical action involving the contents of the atmosphere in normal daylight, an attempt was made, using a Hanovia U.V. lamp, to see whether shorter wave lengths would enhance the process. A sample of freshly distilled acid was divided into two portions, both contained in 150 ml glass beakers. One was exposed to the action of the U.V. lamp placed directly over the surface of the liquid, whilst the other acting as a control was placed in close proximity but shielded from the weak visible light emitted by the lamp by a dark paper jacket. The whole experiment was carried out in a darkroom. Although both beakers were fully open to the atmosphere, the irradiated beaker was shielded from direct, vertically falling particles by the lamp and hence the control was likewise shielded by a metal plate clamped above it. The lamp was left on continuously for 147 hours during which time aliquots were taken at intervals from each beaker and analysed after hydrolysis. The results of this experiment are shown in Fig. 4. The irradiated sample shows an increase both with respect to

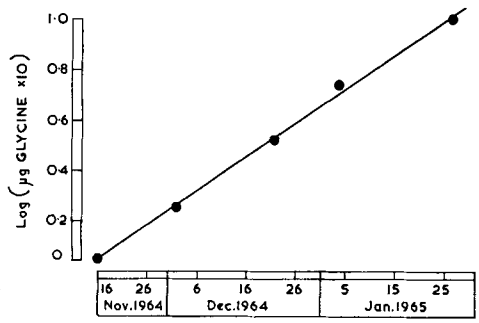

FIG. 3. The linear log. plot of Fig. 2.

the control curve and with time. The overall increase from start to finish is greater in proportion to that obtained in daylight (Fig. 2) for the comparable period of time.

A variety of energy sources have been used for the synthesis of amino acids in the laboratory from simple substances like the system, CO_2–N_2–H_2–H_2O (ABELSON, 1956), and have included electric discharge, U.V. light, α, β, γ and X-rays (MILLER & UREY, 1959) and thermal energy (HARADA & FOX, 1964).

The existence of hydrochloric acid in the atmosphere from ocean derived sodium chloride, appears to have been established by a number of workers (CAUER, 1951; ROBBINS *et al.*, 1959; ERIKSSON, 1960) and the possibility of a relevance to the amino acid content of rainwater may be latent in these observations.

A significant feature of the U.V. irradiation experiment described above was that when aliquots of the acid taken at the end of the experiment from control and test were analysed *without* the hydrolysis procedure no amino acids were detected. Also it was observed that on evaporating the irradiated acid to dryness, a dark residue was formed which was only faintly present in the non irradiated control. The implication of this is that the amino acids found in the acid after exposure to U.V. light or prolonged daylight do not arise directly but from the photochemically formed precursors which under the influence of the prolonged heating during the hydrolysis procedure react to give the amino acids subsequently detected.

The formation of organic material from such simple systems as water and carbon dioxide has been reported, using such widely different energy sources as a Xenon lamp emitting radiation of wavelength 1470A and 1295A (ALTSHULLER, 1958) and high energy ionizing radiation (GARRISON *et al.*, 1951). In general the synthesis of amino acids is considered possible only under reducing conditions (LOWE *et al.*, 1963) but strong acid with its high hydrogen ion concentration may yet provide suitable conditions.

The enhanced amino acid pattern found in rainwater after hydrolysis may in part be in consequence of non amino acid precursors present in the rainwater reacting under the effect of the prolonged heating during hydrolysis in the acid medium.

ROSSBY & EGNER, 1955, investigating the chlorine retention phenomenon in the atmosphere with respect to ocean derived sodium chloride, differentiated four zones extending from the windward coast, inland. Study of the amino acid content of rainwater in relation to these zones may be informative, since the retention of chlorine is synonymous with the formation of atmospheric hydrogen chloride.

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СОДЕРЖАНИЕ АМИНОКИСЛОТ В АТМОСФЕРНЫХ ОСАДКАХ

Определялось с помощью хромографической бумаги содержание аминокислот в дождевой воде за период несколько месяцев. Было установлено наличие продолжительных и широко распространенных процессов, в которых изменялось количество глацина, валина, аланина, глютаминовой кислоты, лейцико-изолейцина.