Reports

Probable Role of Gamma Irradiation in Origin of Life

This article (*1*) reports the production of simple organic compounds, including at least one and possibly two amino acids, from ammonium carbonate by gamma irradiation from a cobalt-60 source. To our knowledge, this is the first instance in which amino acids have been obtained directly from completely oxidized forms of carbon in the absence of free hydrogen. In our opinion, gamma irradiation from terrestrial sources is a much more probable agent than lightning and sunlight for the generation of most of the so-called "organic milieu," the nonliving mixture of carbon compounds which is generally considered to be a prerequisite for the origin of life.

Löb (*2*) in 1913 appears to have been the first to obtain evidence of an amino acid after subjecting CO, NH_3, and H_2O to a silent electric discharge. Miller (*3*) recently greatly extended such experiments by using NH_3, H_2O, H_2, and CH_4; he obtained a wide variety of organic substances. Abelson (*4*) has confirmed this work while using CO and CO_2 in place of CH_4. Calvin and coworkers (*5*) irradiated a variety of simple compounds with helium ions and with ultraviolet light and obtained more complex substances. Quite recently Hasselstrom, Henry, and Murr (*6*) formed amino acids from ammonium acetate by means of an electron accelerator. Oparin (*7*) has proposed that hydrocarbons arose from the polymerization of acetylene which formed from the reaction of water with metallic carbides.

In our experiments, 45-g quantities of ammonium carbonate (lumps, Mallinkrodt A. R.) were sealed under water-pump vacuum (approximately 7 mm Hg) in two glass ampules (outside diameter, 25 mm). Each ampule was irradiated with gamma rays from a 4000-c cobalt-60 source for approximately 15 days to give dosages of 500 ± 15 Mrep. After irradiation, the ampules were opened and subjected to water-pump vacuum while they were immersed in a steam bath. Under these conditions, all ammonium carbonate was vaporized in about 5 hours. Volatile organic compounds also were removed. The residue, approximately 0.2 g, consisted of about equal quantities of a sublimate of large cubical crystals at the top of the ampule and a white powder at the bottom.

Infrared spectra of pressed KBr plates showed the sublimate (m 108° to 110°C uncorrected) to be ammonium formate. The white powder was apparently too complex a mixture to permit any positive identification. Paper chromatography of the powder, however, showed the presence of glycine, ammonium formate, possibly alanine, and an unknown, more slowly moving substance that also gave a positive ninhydrin test. Lack of material did not permit positive identification of alanine. Glycine was identified in five solvent systems: 77 percent ethanol; 80 percent pyridine; phenol buffer at pH_{12}; *sec*-butanol and 3 percent NH_4OH (3/1); and *n*-butanol, acetic acid, and water (4/1/5). In the first four systems, the R_f value for the unknown was identical with that of known glycine. In the last system, the R_f was slightly less than that of pure glycine, but this was shown to be due to the presence of ammonium formate in the unknown. Controls run in the same manner but not irradiated gave a barely discernable residue and negative ninhydrin tests of the water solution.

It is not known whether the massive dose used (500 Mrep) is necessary. The important fact is that amino acids were formed from ammonium carbonate.

Ionizing radiation can, of course, cause the destruction of organic compounds as well as their formation. One can assume, therefore, that organic substances would not concentrate on even a sterile earth unless some great shield were available to protect them once they formed. The seas could be this shield. Inorganic and simple organic compounds on or close to radioactive sources, especially gamma emitters above or below the sea, could be transformed into amino acids. These amino acids, with the help of rain and ocean currents, could migrate to dark, nonradioactive areas where, as suggested by Bernal (*8*), they could be accumulated and oriented by adsorption on clays or quartz (as sand).

There appears to be little doubt that enough radioactivity to accomplish such reactions existed after the earth had cooled sufficiently to permit condensation of the water vapor to form the oceans. An upper limit on the irradiation time may be obtained if it is assumed that the terrestrial radioactivity was the same as it is today. Indeed, if we consider only the three major series (U^{238}, 4.5×10^9 yr; Th^{232}, 13.9×10^9 yr; and U^{235}, 0.7×10^9 yr) (*9*), allowances for decay would not result in significantly greater magnitudes. High-activity ores existing today could deliver the necessary radiation doses in tens of years.

No doubt amino acids are being formed by physical processes even today. However, they or the rest of the physically formed organic milieu can no longer accumulate as they did in a sterile world. The present bios zealously guards its birthright and quickly devours any molecules that could give rise to a contender.

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Variation in Normal Sodium, Potassium, and Calcium Levels in Wistar Albino Rats

In the last decade there has been a marked increase in the use of flame spectrophotometry for physiological studies of electrolyte changes in tissue fluids. As a rule, small numbers of control animals have been used since most studies are predicated on the belief that electrolyte concentrations in the blood normally

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Table 1. Morning-evening and day-to-day comparison of serum ion levels (milliequivalents per liter) in male SF-Wistar rats. All dates are 1955; "CV" represents coefficient of variation.

Expt. No.	Date bled	Time	N	Body wt. ($g \pm \sigma$)	Sodium $\bar{X}$	Sodium σ	Sodium CV	Calcium $\bar{X}$	Calcium σ	Calcium CV	Potassium $\bar{X}$	Potassium σ	Potassium CV
1	6 May	11 a.m.	11	281 ± 31	153.3	1.8	1.2	5.43	0.30	5.5	6.50	0.50	7.7
2	6 May	11 p.m.	11	294 ± 28	156.4	1.4	0.9	5.23	0.18	3.4	5.83	0.50	8.5
3	17 May	11 a.m.	12	195 ± 12	157.3	1.9	1.2	5.14	0.17	3.3	6.02	0.44	7.3
4	17 May	11 p.m.	10	189 ± 14	159.6	2.2	1.4	5.21	0.18	3.4	5.48	0.26	4.7
5 (Total)		11 a.m.	23	236 ± 10	155.4	2.7	1.7	5.28	0.28	5.3	6.32	0.58	9.2
6 (Total)		11 p.m.	21	239 ± 12	157.9	2.4	1.5	5.22	0.18	3.4	5.66	0.43	7.6

remain stable within fairly narrow limits. For some time it has been appreciated that the functions of many organs undergo diurnal variation, but only recently has attention been focused on the fact that marked changes in serum constituents also occur (*1–3*).

This report (*4*) is concerned with flame spectrophotometric determinations of serum sodium, potassium, and calcium levels in normal rats and includes data on day-to-day and diurnal variation of these ions. The first phase of the study involved the determination of the normal range of variation of serum sodium, potassium, and calcium in the Wistar albino rat. Animals of comparable age and weight were ordered from two separate supply houses (designated CF- and SF-Wistar rats) to check on possible differences in ion levels of inbred rat strains that presumably came from the same original stock. Blood samples from CF-Wistar rats were taken from 1 to 3 P.M. each day; SF-Wistar rats were sampled at random intervals mornings and afternoons. All ion analyses were made using the Beckman model DU flame spectrophotometer with photomultiplier attachment. The analytic procedure was that described by Kingsley and Schaffert (*5*). The procedure for collecting tail blood samples is similar to that described by Halberg (*1*), and the routine of preparing serum samples was rigidly standardized to minimum variation due to hemolysis or the influence of exposure of blood to air during collection of samples. The degree of hemolysis was checked with a hand spectroscope.

The distribution curves for all three ions in CF-Wistar rats compared well with hypothetical "normal" curves, the means and medians coinciding in every case, and about 70 percent of the values falling within the mean ± 1 standard deviation. Mean values and standard deviations from 109 serum determinations, in milliequivalents per liter, were as follows: 151.0 ± 4.4 for sodium; 5.54 ± 0.62 for potassium; and 4.93 ± 0.27 for calcium. The coefficients of variation were 2.9, 11.2, and 5.4 percent for sodium, potassium, and calcium, respectively.

Normal distribution curves for the same ions were also obtained in the blood serum of SF-Wistar rats. However, the mean levels of the ions were found to be significantly higher than those in the CF-Wistar group. Mean ion values (milliequivalents per liter) with the standard deviations from 72 serum samples of SF-Wistar rats were as follows: for sodium, 157.2 ± 4.2; for potassium, 6.11 ± 0.56; for calcium, 5.41 ± 0.30. The coefficients of variation were 2.7 percent for sodium, 9.1 percent for potassium, and 5.5 percent for calcium.

It was next decided to establish whether the time of blood sampling is important when serum ion analyses are made. Approximately 48 SF-Wistar male rats were used in this study. A shipment of 24 rats was divided into two equal groups, one to be bled at 11:00 A.M., the other at 11:00 P.M. of the same day. A second shipment of the same strain of rats was obtained a week later, and the same procedure of blood sampling was followed. Table 1 summarizes ion data obtained from 44 serum samples, and Table 2 shows the results of an analysis of the data using Fisher's t test. It was found that serum sodium concentrations were significantly lower at 11:00 A.M. than at 11:00 P.M. Conversely, potassium levels were higher in the morning. Mean levels of calcium, on the other hand, were not affected by taking blood at different times of the day.

It was further found that the levels of serum sodium from rats bled on 17 May 1955 were significantly higher, and the potassium concentrations lower, than the levels of these ions in rats bled at corresponding times on 6 May 1955. The results suggest that day-to-day fluctuations in serum ions may also be present. Similar results were obtained for serum sodium and potassium when ion data of CF-Wistar rats was analyzed on a day-to-day basis. Serum sodium values of 24 rats bled on four separate days at weekly intervals gave means plus or minus standard errors as follows: 152.0 ± 0.9, 146.6 ± 0.6, 149.0 ± 0.5 and 154.8 ± 0.7 milliequivalents/lit. Corresponding potassium levels on the same days were 5.59 ± 0.08, 5.77 ± 0.12, 5.55 ± 0.12 and 5.27 ± 0.16 milliequivalents/lit.

Statistical analysis of the data on CF-Wistar rats by separation into sexes and weight groups showed no significant differences in serum ion levels of male and female rats.

The extent of variation in ion concentrations in the CF-Wistar strain of rats is similar to that reported by Elliott and Holley (*6*) in a study of 400 normal human subjects. Values reported by these investigators were used as an index of expected variability because a search of the literature failed to bring to light any flame photometric studies of normal ion variation in laboratory animals. The mean sodium and potasium values of CF-Wistar rats are somewhat higher than those reported by Albritton (*7*) but agree closely with values reported by Bernstein (*8*). The SF-Wistar strain, however, showed consistently higher sodium and potassium values, suggesting the presence of intraspecific strain differences in the levels of these ions.

The data seem to indicate that a part of the serum ion variability, especially with sodium and potassium, may be the result of diurnal variations of these ions. Likewise, day-to-day variation in ion levels must also be considered as a possible factor contributing to normal ion variability. It should be recalled in this connection that diurnal fluctuations in other serum constituents have already been amply demonstrated, notably with respect to leucocytic blood components, serum iron, hemoglobin, and serum proteins (*1, 3, 9*). With the greater precision now possible with current spectrophotometric techniques, it should not be surprising to find diurnal variations in serum electrolytes.

Although no conclusions could be drawn from the present study about the mechanisms underlying diurnal or daily ion variation, it was felt that the ob-

Table 2. Statistical analysis of serum ion data; P, probability; d.f., degrees of freedom. The numbers in column 1 refer to the experiment numbers in column 1 of Table 1.

Comparison	Sodium t	Sodium d.f.	Sodium P	Calcium t	Calcium d.f.	Calcium P	Potassium t	Potassium d.f.	Potassium P
Experiments 1 and 2	4.32	20	< 0.01*	1.80	19	≈ 0.10	3.06	20	< 0.01*
Experiments 3 and 4	2.51	20	≈ 0.02*	0.89	20	≈ 0.40	3.31	19	< 0.01*
Experiments 5 and 6	3.14	42	< 0.01*	0.83	41	≈ 0.40	4.20	41	< 0.01*
Experiments 1 and 3	5.03	21	< 0.01*	2.74	21	< 0.01*	2.61	21	≈ 0.02*
Experiments 2 and 4	3.77	19	< 0.01*	0.24	18	> 0.50	2.00	18	≈ 0.05*

* Significant using Fisher's t test.

served changes could not be ascribed to either technical errors or to disturbances of rats by extraneous laboratory noises. Potential errors in analytic technique were eliminated by routinely running calibration checks on freshly prepared standards. Noise was eliminated as a factor on the basis of other experiments in our laboratory where no differences were found between ion levels of rats that were exposed to noises approximating jet-engine intensity levels and ion levels of nonexposed controls. In the latter studies, significant day-to-day fluctuations in serum sodium and potassium occurred although the means, standard deviations, and coefficients of variation were the same in noise-exposed and control rats bled at corresponding times on any one day.

The net effect of these investigations points to the need for considering diurnal and day-to-day variations in serum ions when dealing with electrolyte changes in animals. Rigid standardization of the time of sampling is mandatory in experiments when small numbers of animals are used to establish "normal" ion levels and when the interpretation of electrolyte shifts is predicated on the assumption that such levels represent a stable base line.

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Preparation of Growth Hormone from Pituitaries of Man and Monkey

Despite occasional suggestions of effectiveness (*1*), growth hormone prepared from the pituitaries of slaughterhouse animals has thus far been generally ineffective in man. Preliminary tests by Beck and Venning (*2*) have shown pronounced anabolic effects in man with growth hormone extracted from human and from simian pituitaries, and we wish to record the method of preparation of the materials used in these tests (*3*). The preparation from monkey was also found by Knobil, Wolf, and Greep to be effective when it was tested in monkeys, although bovine and porcine preparations were inert in that species (*4*).

Table 1. Weight yield of various fractions during preparation of growth hormone from pituitaries of three species. Human and monkey pituitaries were used as obtained, without removal of posterior lobe; the pig preparation was made from anterior lobes only. The values cited were obtained from human pituitary batches of 11.25 g and 24 g of acetone-dried powder, two batches of monkey pituitaries (20.8 and 13 g of powder) and many batches of pig pituitaries.

	Man		Monkey	Pig
	Batch 1 (%)	Batch 2 (%)	(%)	(%)
Acetone-dried pituitary powder	100	100	100	100
Acetone precipitate from glacial acetic extract	4.5	5.4	7.2	7.0
Ether precipitate—"crude extract"	9.4	13.2	20.6	12.0
Adsorbed by oxycel—"corticotropin fraction"	0.16	0.26*	0.14	0.24
Second oxycel adsorption	0.12	0.4*	0.26	0.20
Precipitate at *p*H 8.5	3.12	4.6	7.2	~7.0
Alcohol precipitate—"growth hormone"	3.1	4.6	3.0	1.5

* Double amounts of oxycel were used with batch 2.

The human pituitaries were collected post-mortem and stored in acetone after the amount necessary for microscopic study had been removed (*5*). The activity of the growth hormone survived the delay between death and autopsy, in agreement with observations on the survival of activity in animal pituitaries (*6*). The pituitaries from monkeys were collected in the laboratories of several pharmaceutical companies from rhesus monkeys used in the preparation of poliomyelitis vaccine and were stored frozen (*7*).

Acetone-dried pituitary powder was prepared by homogenization in acetone with a Virtis 45 or Waring Blendor, further washing with acetone on a sintered-glass filter, and desiccation in a vacuum. Growth hormone was extracted and purified by the method originally devised for porcine glands (*8*). The procedure involved extraction with glacial acetic acid at 70°C, removal of an acetone precipitate, precipitation of a crude fraction with ether, removal of corticotropin and intermedin from a weak acetic acid solution with 11 percent COOH oxycellulose (*9*), removal of a *p*H 8.5 precipitate, and precipitation of growth hormone with ethyl alcohol. The weight yield from pituitaries of man and monkey was greater than the yield from pituitaries of pig (Table 1). The activity per unit weight as assayed in the hypophysectomized rat was approximately the same for the three species, with the human material perhaps slightly more active, and the monkey slightly less active, than the pig. It is assumed that the product is chemically impure, in view of the recent finding that animal growth hormone prepared by this and other methods can be further fractionated chromatographically (*10*).

Certain features of this method of preparation of growth hormone made it particularly suitable for its present use. Storage of the human pituitaries in acetone simplified the collection of glands, treatment of the pituitary powder with acetone, ether, and hot glacial acetic acid provided strong bactericidal and viricidal action in the extraction of human pituitaries of indeterminate origin, and the virtual absence of thyrotropin, as well as the low degree of contamination with other pituitary hormones, made the final product well suited for clinical use.

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