such as the decrement found in prolonged search for infrequent signals, would be the result of the reinforcement schedules involved. In addition, it should be possible to develop and adopt schedules that would mold the observing behavior into the form desired for practical purposes, making possible the engineering of monitoring and inspection tasks so that the operator's behavior is molded in a fashion that would provide superior man-machine systems.

JAMES G. HOLLAND
Engineering Psychology Branch, U.S. Naval Research Laboratory, Washington, D.C.

References and Notes

1. B. F. Skinner, *The Behavior of Organisms* (Appleton-Century, New York, 1938).
2. For a similar concept of observing responses with animals see L. B. Wyckoff, *Psychol. Rev.* 59, 431 (1952).

19 December 1956

Synthesis of Amino Acids by Beta Radiation

A recent paper (*1*) from this laboratory described a method of synthesizing oxalic acid by subjecting aqueous inorganic bicarbonate solutions to ionizing radiation. Acetic acid and inorganic acetates in aqueous solutions, upon irradiation, produce di- and tricarboxylic acids (*2*). The present investigation shows that ammonium acetate, on exposure to β-radiation in water solutions, forms, in addition to di- and tricarboxylic acids, small amounts of glycine, aspartic acid, and an unknown amino acid believed to be diaminosuccinic acid. Glycine would be the initial amino acid formed, whereas both of the aminosuccinic acids would be secondary ones. This is postulated because of the ease of formation of succinic acid and its homologs from the acetic acid moiety; that is, the active site is the alpha hydrogen to the carboxyl group.

The following sequence of reactions may be applicable to the formation of the observed products. Ammonium salts of organic acids, being salts of weak acids and bases, undergo hydrolysis reactions in the presence of water.

$$CH_3COONH_4 + H_2O \rightarrow NH_4OH + CH_3COOH \quad (1)$$

$$NH_4OH \rightarrow NH_3 + H_2O \quad (2)$$

Irradiation of aqueous solutions of ammonium acetate produces the following reactions (*3*).

$$NH_3 \text{—}\wedge\wedge\wedge\wedge\rightarrow NH_2\cdot + H\cdot \quad (3)$$

$$H_2O \text{—}\wedge\wedge\wedge\wedge\rightarrow HO\cdot + H\cdot \quad (4)$$

$$CH_3COONH_4 + OH \rightarrow H_2O + \cdot CH_2COONH_4 \quad (5)$$

Combining Eqs. 3, 4, and 5, one obtains

$$\cdot CH_2COONH_4 + \cdot NH_2 \rightarrow NH_2CH_2COONH_4 \text{ glycine salt} \quad (6)$$

$$2 \cdot CH_2COONH_4 \rightarrow \begin{matrix} CH_2\text{-}COONH_4 \\ | \\ CH_2\text{-}COONH_4 \end{matrix} \quad (7)$$

$$\begin{matrix} CH_2\text{-}COONH_4 \\ | \\ CH_2\text{-}COONH_4 \end{matrix} + \cdot OH \rightarrow \cdot CHCOONH_4 + H_2O \quad (8)$$
$$\qquad\qquad\qquad\qquad\qquad\qquad\quad |$$
$$\qquad\qquad\qquad\qquad\qquad\qquad CH_2COONH_4$$

$$\cdot NH_2 + \cdot CHCOONH_4 \rightarrow NH_2CHCOONH_4 \quad (9)$$
$$\qquad\qquad\quad |$$
$$\qquad\quad CH_2COONH_4$$

aspartic acid salt

A sequence of reactions similar to those of Eqs. 8 and 9 may also be written to postulate the formation of diaminosuccinic acid.

Two concentrations, 1 and 2.5 percent, of ammonium acetate were exposed to various dosages of β-radiation by means of a 2-Mev van de Graaff electron accelerator. The solutions were analyzed for amino acid content by a modification of Levy's (*4*) method for quantitative paper chromatography determination by measuring the absorbancy of the material eluted from the spots (*5*).

In the irradiation of ammonium acetate solutions, two aqueous concentrations of ammonium acetate (Merck reagent) were employed—namely, 10.002 g of ammonium acetate per liter and 25.006 g/lit. Approximately 200 ml of each of these solutions was placed in heat-sealed polyethylene bags. Six bags of each solution were subjected to β-radiation at 2, 10, 20, 30, and 50 Mrep in a van de Graff electron accelerator.

In order to separate amino acids from nonamino acids, 143 ml of wet Dowex-50 resin, 20–50 mesh, in the acid form, was poured into a glass tube 42 mm wide and 240 mm long. The resulting column or bed of resin was treated with 500 ml of 2*M* ammonium hydroxide, then with 500 ml of distilled water, and then with 500 ml of 2*M* hydrochloric acid. The column was then washed with distilled water until the effluent reached the *p*H of the distilled water.

Two hundred milliliters of the irradiated solution was pipetted into a separatory funnel above the column and allowed to drop slowly into the column as solution was withdrawn from the bottom. When addition was complete, the column was washed with approximately 300 ml of distilled water or until the effluent was of the same *p*H as the tap water. It was demonstrated with a known mixture that acetic and succinic acids were recovered quantitatively in a total volume of 500 to 600 ml of water, including that in which the sample was dissolved. This effluent was set aside for analysis for the nonamino acids.

The amino acids were displaced from the column by washing with 500 ml of 2*M* ammonium hydroxide. The effluent was collected in 10-ml fractions, and aliquots of the fractions were spotted on Whatman No. 1 filter paper and heated for 5 minutes to drive off ammonia. The paper was sprayed with ninhydrin solution (50 ml of 0.1-percent ninhydrin in ethanol, 2 ml of collidine, and 15 ml of glacial acetic acid). The ninhydrin-positive fractions were combined, care being taken to transfer the solutions quantitatively.

The solution containing the amino acids was evaporated to dryness at room temperature in a Roto-Vap. The residue was dissolved *in situ* in 5 ml of a 2-percent sodium bicarbonate solution. To this solution was added 10 ml of alcohol containing 0.101g of 2,4-dinitrofluorobenzene. The total volume of solution was 14.8 ml. A bright yellow solution resulted immediately. The flask was swirled from time to time to insure complete contact of the solution with an insoluble residue in the flask that was assumed to be from the ion-exchange resin. This residue does not contain amino acid, since 99.5 percent of a known mixture of amino acids was recovered when it was carried through the entire analytic procedure. In this run, the residue was still present after the reaction with 2,4-dinitrofluorobenzene was complete. The solution was

Table 1. Amino acids resulting from the β-irradiation of aqueous ammonium acetate.

Dosage (Mrep)	Quantity of amino acid from 2.5% sol'n. (μg)			Quantity of amino acid from 1% sol'n. (μg)		
	Glycine	Aspartic acid	Unknown*	Glycine	Aspartic acid	Unknown*
2	†					
10	33	23				
20	658	266		672	422	125
30	1400	985	360	1030	940	361
50	2200	1872	550	1720	1850	1079

* Unknown calculated as diaminosuccinic acid using $E_{365} = 15{,}500$.
† Where blanks appear, the quantity of amino acid was not measurable by the method employed.
k glycine = 1.03; k aspartic = 0.99; k unknown = 1.000 (calculated as diaminosuccinic acid).

allowed to stand overnight to insure complete reaction. Good results were obtained on known mixtures after a reaction time of only 2 hours.

Strips of Whatman No. 54 filter paper, 7⅜ by 18 in., were soaked in 0.05*M* phthalate buffer and allowed to dry. The strips were ruled with lines parallel to the long edges, the first 11/16 in., and then four at intervals of 1½ in. Of the four pathways so marked, three were used for samples and one was left as a blank. The samples were applied at spots 4 in. from the upper end of the strip, thus clearing the antisyphon rod that supported the paper in downward development. Each spot contained the amino acid from two or three 7.1-μlit aliquots.

The chromatogram was developed for 18 hours by the descending method with t-amyl alcohol that had been equilibrated with the phthalate buffer. The chromatogram was covered with brown paper to minimize the losses of light-sensitive derivatives.

After development was complete, portions of the paper containing the spots were excised and transferred to 6-in. test tubes. Blanks were similarly cut from the appropriate track at the same distance from the starting line as the unknown. In order to insure standardization of the size of the piece of paper excised, a template was fashioned from a thin sheet of Plexiglas. Spots of the 2,4-dinitrophenol and 2,4-dinitroaniline were not excised. Four milliliters of water was pipetted into each tube, and the samples were allowed to stand for 2 hours in the dark with occasional agitation.

The extract was transferred to a 1-cm glass cell, and its absorbancy was taken against water in a Beckman DU spectrophotometer at 365 mμ with a slit width of 44 μ. The results appear in Table 1. Calculations were made from the following formulas:

$$\frac{(\text{Absorbancy})\,(k)}{15.6} = \mu\text{moles/ml of eluate}$$

where k is an empirical constant for each amino acid determined by Levy,

$$\frac{(\mu\text{mole/ml})\,(4\text{ ml})\,(1000/\text{ml})\,(14.8\text{ ml})}{\text{Number spotted}} = \mu\text{moles of acid}$$

$$(\mu\text{mole})\,(\mu\text{g}/\mu\text{mole}) = \mu\text{g of amino acid in 200 ml of irradiated sample}$$

Several chromatograms were run with 1.5*M* phosphate buffer as the developing solvent. This buffer is prepared by making a solution 1.0*M* in NaH_2PO_4 and 0.5*M* in Na_2HPO_4. Development was allowed to proceed about 4 hours. The spots of the unknown) diaminosuccinic acid) and aspartic acid were excised and extracted, and the absorbancy was taken. Glycine did not separate from dinitrophenol. In this way, a partial check of the first results could be made.

Blank runs on ammonium acetate solutions that had not been exposed to β-radiation revealed no amino acids.

TORSTEN HASSELSTROM
MALCOLM C. HENRY
BROWN MURR

U.S. Army Quartermaster Research and Development Command, Natick, Massachusetts

References and Notes

1. T. Hasselstrom and M. C. Henry, *Science* 123, 1038 (1956).
2. W. M. Garrison *et al.*, *J. Am. Chem. Soc.* 75, 2459 (1953); T. Hasselstrom and M. C. Henry, paper presented at the ACS meeting in Dallas, Tex. (Apr. 1956).
3. For Eq. 3, see T. Rigg, G. Scholes, J. Weiss, *J. Chem. Soc.* 1952, 3034 (1952); for Eq. 4, see M. J. Day and G. Stein, *Nucleonics* 8, 37 (1951).
4. A. L. Levy, *Nature* 174, 126 (1954).
5. A report on the composition of the nonamino acid portion is in preparation.

13 November 1956

Cholinesterase in the Nemertean Prostoma rubrum

In conjunction with studies on various invertebrates collected in our vicinity, experiments were undertaken to determine the concentration of cholinesterase in the invertebrates. In the series of studies, it was found that the fresh-water nemertean *Prostoma rubrum* has a high concentration of cholinesterase in its body tissues. A more detailed study was made to determine whether "specific" or "nonspecific" cholinesterase was present in the animals.

Prostoma rubrum was collected from Mill Pond, Tooele County, Utah, in October 1955 and 1956. This animal is found abundantly only during the fall. Large numbers of individuals were isolated and kept in white enameled pans and petri dishes in the laboratory. The animals measured up to 15 mm in length, and their average weight was 1.5 mg.

Assays for cholinesterase activity were performed on groups of 15 to 40 animals. The animals were rinsed in spring water, blotted dry with tissue paper, and weighed. They were then homogenized in physiological saline with a motor-driven Potter-Elvehjem homogenizer. The homogenates were diluted to a total volume of 10 ml and kept overnight in the cold room. They were assayed on the following day. Cholinesterase activity was determined by the titrimetric method. Acetylcholine bromide (ACh), acetyl-β-methylcholine chloride (MeCh), and butyrylcholine chloride (BuCh) were used as substrates for these assays, which were run at 25°C. Final substrate concentrations of 0.005*M* acetylcholine bromide, 0.01*M* acetyl-β-methylcholine chloride, and 0.01*M* butyrylcholine chloride were used for these assays.

The results of these experiments are given in Table 1, where they are recorded in terms of cholinesterase activity (Q_{ChE}, in milligrams of substrate hydrolyzed per 100 mg of tissue, per hour). Average cholinesterase activities of 712, 658, and 515 were obtained for homogenates of the first three groups of animals, the homogenates being kept in the cold room overnight, except the homogenate from the last group of animals, which was assayed immediately after it had been homogenized. Samples of these homogenates were kept in the cold room for 4 additional days and were subsequently assayed for cholinesterase activity. The stored homogenates showed a great increase in activity—the range at this time was from 899 to 676.

The results obtained by the titrimetric method were checked by the colorimetric method on homogenate that had been frozen for 2 months. The cholinesterase activity obtained by this method was 510, compared with an activity of 528 obtained by the titrimetric method.

Assays were performed on homogenates of three groups of animals to determine the presence or absence of specific and nonspecific cholinesterases in the nemertean. Aliquots of homogenates were assayed, and it was found that the average cholinesterase activities for these three groups were 525 (acetylcholine bromide substrate), 272 (acetyl-β-methylcholine substrate), and 125 (butyrylcholine substrate). These results show that both specific and nonspecific cholinesterases are present in *Prostoma rubrum*.

The occurrence of cholinesterases has

Table 1. Cholinesterase activity (Q_{ChE}) in *Prostoma rubrum*. The substrates were acetylcholine bromide (ACh), acetyl-β-methylcholine chloride (MeCh), and butyrylcholine chloride (BuCh).

No. of animals	Total wt. (mg)	Q_{ChE} (mg of substrate hydrolyzed/100 mg of tissue/hr)		
		ACh	MeCh	BuCh
20	29.4	712 899*		
20	34.6	658 776*		
40	51.4	515 676*		
20	34.2	582	282	123
15	18.6	539	263	
20	28.8	454	272	127
–	708.0	528		
–	708.0	510†		

* Stored 5 days. † Colorimetric method (frozen 2 months).