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TRACER STUDIES OF THE UPTAKE
OF ORGANIC COMPOUNDS BY PLANARIANS

Edward L. Bennett, Marie Hebert, and Ann M. Hughes

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TRACER STUDIES OF THE UPTAKE OF ORGANIC COMPOUNDS BY PLANARIANS*

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ABSTRACT

The uptake of nucleic acid precursors, amino acids, organic acids, and carbohydrates from aqueous solution by planarians has been investigated. Of these various classes of compounds, the organic acids were taken up most rapidly and extensively. The nucleic acid precursors, adenine and cytosine, were also effectively used. The more polar compounds such as the amino acids and carbohydrates were less well utilized. The distribution of these compounds between high- and low-molecular-weight compounds was also studied. Radioactivity was transferred and retained when labelled planarians were cannibalized by D. tigrina. Radioautographs of planarians labelled with propionate and adenine showed that incorporation was general throughout.

INTRODUCTION

At this Symposium it is superfluous to review either the numerous theories of memory or the interest that has existed, and for many still exists, in planarians as a useful tool in studies of chemical processes which occur in learning. Because of this interest in planarians, a few years ago we attempted to train planarians reliably and reproducibly. At the same time we studied the uptake of a number of organic compounds by planarians. Our objective was not to investigate the intermediary metabolism of these compounds in detail, but rather to determine in a general way (1) the relative efficiency with which different classes of organic compounds were utilized, (2) the variation within a class of the degree of utilization, and (3) some variables which influence uptake. The parameters affecting uptake which we studied included the external concentration of the organic compounds, and the time of contact. In addition, the rate of loss of the label when the planarian was removed from the tracer solution, and the distribution of the utilized chemical between "soluble" and "insoluble" compounds were determined. The retention and distribution of the label when transferred by cannibalism were also investigated. Radioautographic studies were carried out to confirm the uptake of the tracer compounds and to investigate their distribution among cells of the planarian.

Our ultimate objective, which we did not attain, was to demonstrate that labelled macromolecules transferred by ingestion or injection would subsequently reach the neural tissue of the recipient planarian. Many of us would probably be much more receptive to the notion that memory can be transferred if it could be conclusively shown that neural material from the donor was transferred to neural tissue of the recipient. The recent electron microscope studies of the

neural structure of planarians by Morita and Best,¹ which have been presented at this Symposium, illustrate some of the problems to be surmounted before transfer of neural components to neural tissue can be demonstrated in planarians.

In our studies, we compared the uptake of (a) purines, pyrimidines, and nucleosides, (b) amino acids, (c) fatty acids, and (d) carbohydrates. Of these compounds, fatty acids are taken up most extensively. Butyrate-2-¹⁴C and valerate-2-¹⁴C are taken up more rapidly than the lower fatty acids, and form at least four or five major compounds and about 10 minor compounds. Propionate-2-¹⁴C, which is also extensively utilized by planarians, is lost only slowly and is transferred to and retained by cannibals. Of the nucleic acid precursors tested, adenine and cytosine exhibited the greatest uptake. Adenine was converted to 5'-adenylic acid and its derivatives and to nucleic acid adenine and guanine. The amino acids and carbohydrates, as well as inorganic phosphate, were taken up comparatively slowly.

METHODS

Typically, the planarians Dugesia dorotocephala or D. tigrina were placed in a radioactive solution contained in a 10- or 20-ml beaker and stored in a dark cupboard. The "concentration" of the planarians was three to four per ml of solution, and the concentration of the radioactive compound varied from 1.0 to 10 $\mu\text{M}/\text{ml}$. A local spring water (Alhambra) was used, and the pH was adjusted to 7 after the compound was dissolved.

The amino acids, organic acids (as sodium salts), glucose, and sucrose had been prepared in our laboratory. Each compound had approximately 0.5 to 1 mCi/mM and was used without further dilution. Purity was checked prior to use by paper chromatography and radioautography. The valine sample had 10 to 15% of an unidentified impurity present. The nucleic acid precursors were commercial samples and were diluted when used at 2.5 $\mu\text{M}/\text{ml}$ to approximately 3 mCi/mM for the ^{14}C -containing compounds or 20 mCi/mM for the ^3H -containing materials. At 10 $\mu\text{M}/\text{ml}$, the specific activities were further reduced by a factor of two by addition of appropriate concentrations of carrier. Concentrations were then determined by spectrophotometric methods. About 40% of the radioactivity of the adenine- ^3H sample was volatile when a solution was evaporated to dryness. Data obtained with this sample were corrected accordingly. No similar check was made of the other ^3H -containing samples. Prior to analysis, a planarian was removed from the radioactive solution and passed through three successive rinses of water in a spot plate. The following basic fractionation procedures were followed:

1. Trichloroacetic Acid (TCA) Method

An individual planarian was homogenized in 500- μl of distilled water by use of a micro Teflon-glass homogenizer. After 25 μl of the homogenate was removed for a determination of the total radioactivity present, 100 μl of 50% TCA was

added to the remainder of the homogenate, and the mixture was centrifuged. The precipitate was washed twice with 500 μ l of 10% TCA, and the activity of the combined supernatants (TCA-soluble fraction) was determined by counting a 100- μ l aliquot. In some experiments, the TCA was removed by continuous ether extraction, and the activity in the aqueous phase was redetermined. The residue in the homogenizer was extracted twice with 500 μ l of 95% ethanol (this step was eliminated in some preliminary experiments) and then dissolved by warming overnight at 37°C with 50 μ l of 1N KOH. The KOH solution was then diluted to 500 μ l and heated to 80°C for 2 to 3 h. Aliquot portions were taken of the ethanol extracts and the KOH-hydrolyzed residue for determination of radioactivity. An approximate measure of the size of the worm was usually made by determining the protein content of an aliquot of the KOH-soluble residue by means of the Folin-Wu color reaction.² The results were expressed in terms of m μ M uptake: per 100 mg protein or 1 mg planarian (a planarian was assumed to 10% protein, and those used normally weighed 2 to 4 mg).

2. Ethanol Extractions

In a later series of experiments, the fractionation procedure was patterned after that commonly used in photosynthesis experiments. The planarian was homogenized and extracted twice with 500 μ l of 80% ethanol-20% H₂O at 50°C, then twice with 500 μ l of 30% ethanol-70% H₂O. In one series of experiments acetone-water mixtures were also used with similar results. The residue was dissolved by warming with 50 μ l of 1N KOH or by heating overnight at 80°C with 100 μ l of 6N HCl. In either case, the solution was diluted to 500 μ l with H₂O prior to sampling. No protein determinations were made after acid hydrolysis.

Scintillation counting (Tri-Carb--Packard Instrument Co.) was used to determine radioactivity. The scintillator solution consisted of 5.0 g of

2,5-diphenyl oxazole (PPO), 100 mg of 1,4-bis-[2-(5-phenyloxazolyl)] benzene (POPOP), 50 g naphthalene, 400 ml of p-dioxane, 250 ml of ethanol, diluted to 1 liter with toluene. Normally, samples were counted shortly after addition to the scintillator solution in order to minimize a small (maximum 10%) decrease in activity due to settling of particulate material in some samples. The basic protein hydrolysates were neutralized in the counting vial by the addition of HCl. The efficiency was routinely determined by addition of toluene- ^{14}C (or toluene- ^3H in the experiments with tritiated compounds) and was normally 60 to 65% for ^{14}C and 10% for ^3H .

Extracts from the planarians were chromatographed two-dimensionally on Whatman No. 1 filter paper in phenol-water and then in 40% butanol-25% propionic acid-35% H_2O (wt %). Radioactive compounds were located by radioautography with Kodak x-ray film, and the distribution of radioactivity was estimated by counting the principal radioactive areas with a Mylar-window GM tube. Carrier amino acids were located by spraying with ninhydrin. In experiments with nucleic-acid precursors, 60% propanol-30% ammonium hydroxide-10% H_2O (by volume) was substituted for phenol- H_2O , and the location of carrier compounds was determined under uv light.

To test for radioactivity transfer by cannibalism, planarians were labelled for two days in experiment 1 with either adenine- ^3H with a specific activity of 12 mCi/mM or propionate-2- ^{14}C with a specific activity of 0.8 mCi/mM and a concentration of 10 $\mu\text{M}/\text{ml}$. It was estimated that the cannibalized planarians contained 400,000 dpm of ^3H or 150,000 dpm of ^{14}C . In experiment 2, labelling was carried out for three days with propionate-2- ^{14}C and then the donor planarians were placed in water for four days.

For the cannibal experiments, radioactive D. tigrina were cut transversely into four approximately equal segments, and each segment was cannibalized by

an unlabelled, hungry D. tigrina in a 3-in petri dish containing 25 ml of water. Only an approximate estimate of the radioactivity fed could be made by determination of the radioactivity in similar uncut worms at the time of feeding. The time to attack and ingest the victim was observed and was typically about 10 minutes. In about 15% of the cases, the cannibal refused to eat and had to be replaced.

For the histological studies, planarians were first rinsed in several changes of water, as had been done for the chemical-fractionation procedure. They were then fixed in 10% neutral formalin, embedded in paraffin, and serially sectioned at 5 μ . After being placed on slides, the planarian sections were deparaffinized and hydrated. Slides were then dipped in Eastman NTB₂ emulsion, air-dried, and stored in light-tight boxes in the refrigerator for 3 to 4 weeks. At the end of this time, the radioautographs were developed in Kodak D₁₉ for 6 min, rinsed briefly in distilled water, and fixed in sodium thiosulfate. Slides were washed for 1h in running distilled water, then air-dried overnight. The standard hematoxylin-eosin method was used for poststaining the sections.

RESULTS

A. Uptake of Nucleic-Acid Precursors

One of the first experiments undertaken investigated the effect of concentration and time on the uptake of several nucleic-acid precursors--adenine-8- ^{14}C , cytosine- ^3H , and thymine-2- ^{14}C . The results for adenine, shown in Fig. 1, clearly indicate that within the range investigated, uptake was approximately proportional to the external concentration and was also time-dependent. Adenine taken up by the planarians was retained for long periods--the total radioactivity decreased by only one-half over a 3-week period, even though the planarians had been fed brine shrimp every 3 or 4 days after removal from the adenine solution (Fig. 2).

The uptake of several nucleic acid components is compared in Fig. 3. In terms of total uptake, adenine is taken up 3 to 5 times more than the pyrimidine bases uracil-2- ^{14}C and cytosine. The relative uptake of the nucleosides--uridine-2- ^{14}C , cytidine- ^3H , and adenosine- ^3H --is even less. In another experiment, the uptake of thymidine was also shown to be much less than that of adenine.

So far in this discussion, we have emphasized total uptake and retention only, but in these and other experiments the planarians were fractionated into a trichloroacetic acid-soluble (TCA-soluble) fraction, and a trichloroacetic acid-insoluble (TCA-insoluble) fraction. The results were summarized in terms of μM of precursor incorporated into the TCA-insoluble fraction, presumably RNA and DNA. The percentage of the total activity in the planarians which is incorporated in this fraction is given in Table I. When expressed in this manner it appears that cytidine and adenine have comparable efficiencies as precursors of nucleic acid in planarians when the external concentration is about $2.5 \mu\text{M}/\text{ml}$. The superiority of cytosine as a precursor of nucleic acid is even more obvious

when we compare the data obtained at an external concentration of 10 $\mu\text{M}/\text{ml}$ (Fig. 4). This apparent discrepancy in terms of total incorporation is merely a reflection of the size of the soluble nucleotide pool. It is well known that in higher mammals the soluble adenylic acid pool is much larger than that of other nucleotides. Indeed, we have shown that in planarians more than 50% of the TCA-soluble radioactive material after adenine uptake can be accounted for as AMP and its derivatives, together with small quantities of the guanylic acid derivatives. Much of the remainder is hypoxanthine and adenine. Total adenine incorporation in the insoluble fraction continues to increase as precursors for nucleic acids are drawn from the soluble pool. On the other hand, the radioactivity from cytosine in nucleic acids decreases more rapidly than that from adenine. This is a reflection of the small pool size of soluble cytosine derivatives as compared to the large pool size of the soluble adenine compounds.

Of the nucleic-acid precursors studied, cytosine appears to be the best for obtaining the maximum proportion of label in the nucleic acids, and the minimum possible in soluble nucleotides and other low-molecular-weight compounds. These low-molecular-weight compounds would only confuse the interpretation of the fate of RNA during studies of cannibalism or regeneration.

B. Uptake of Amino Acids

The uptake of five amino acids--glycine-2- ^{14}C , DL-serine-3- ^{14}C , DL-alanine-2- ^{14}C , L-leucine-3- ^{14}C , and L-valine-4,4'- ^{14}C --were investigated. The uptake of each of these compounds increased with time up to 2 days and was 1 $\mu\text{M}/100 \mu\text{g}$ of protein at 1 day in the TCA-soluble fraction when the external concentration was 10 $\mu\text{M}/\text{ml}$ (Fig. 5). This value increased at 2 days to 1.5 to 2.0 $\mu\text{M}/100 \mu\text{g}$ planarian protein. Depending somewhat on the time chosen for

comparison, the decreasing order of uptake is alanine, serine, glycine, leucine, and valine.

The uptake into TCA-insoluble material is shown in Fig. 6. The uptake of serine, glycine, alanine, and leucine is in the range from 1.0 to 1.5 $\mu\text{M}/100 \mu\text{g}$ of planarian protein at two days. Valine is taken up less efficiently. After removal of the external radioactive substances, the fraction incorporated into TCA-precipitable material increased as the activity in the acid-soluble pool decreased.

Another experiment indicated that the uptake of glycine was approximately proportional to the external concentration in the range from 1.0 to 10 $\mu\text{M}/\text{ml}$ (Fig. 7). No further investigations were made of the types of compounds into which the amino acids were incorporated.

C. Uptake of Fatty Acids

Of the compounds tested, the fatty acids were taken up most extensively and rapidly. The uptake increased with increasing molecular weight from formate through valerate (Fig. 8). The distribution of radioactivity in planarians kept in formate- ^{14}C , propionate-2- ^{14}C , and valerate-2- ^{14}C was very similar. Approximately 50 to 60% of the radioactivity was TCA-soluble, 10 to 20% of the radioactivity was extracted by ethanol, and approximately 25 to 35% of activity was both TCA- and ethanol-insoluble. From planarians maintained in acetate-2- ^{14}C , 40% of the activity was extracted by cold TCA, 25% by ethanol, and the remainder was insoluble. The distribution of radioactivity did not change markedly up to 1 week after removal of the planarians from the radioactive solutions.

Some preliminary investigation was made of the type of compounds into which these acids were incorporated. Only 10 to 20% of the TCA-extractable

material was subsequently removed by continuous liquid-liquid extraction with ether. This indicates that only a small amount of the activity was present as fatty acids. Inasmuch as the planarians were much more radioactive after soaking in butyrate or valerate, more compounds could be detected by radioautography after paper chromatography of the extracts than from the lower fatty acids. Figure 9 shows a radioautograph obtained from the TCA-soluble fraction of planarians after soaking in valerate for 3 days. Glutamic acid, glutamine, aspartic acid, and alanine appear to be the main radioactive compounds formed. The large amount of radioactivity remaining at the origin yielded primarily glucose after hydrolysis with HCl. Similar patterns, with not as many minor spots detectable, were obtained from "acetate," "propionate," and "butyrate" worms.

D. Uptake of Glucose and Sucrose

Glucose-U- ^{14}C and sucrose-U- ^{14}C were taken up about equally but were relatively little utilized by D. tigrina. The incorporation from glucose into the 10% TCA-soluble fraction was about 1 $\mu\text{M}/\text{mg}$ planarian, or about one-fourth that found with acetate; the incorporation into the fraction subsequently extracted by ethanol was less than 0.5 $\mu\text{M}/\text{mg}$, and less than 1 $\mu\text{M}/\text{mg}$ was TCA-insoluble. Sucrose gave about four times as much incorporation into the TCA-soluble fraction, but only slightly more incorporation than glucose into the ethanol-soluble and the residue fractions.

E. Uptake of Phosphate

Only one experiment was done to investigate the uptake of phosphate. In this experiment phosphate uptake was found to increase with external concentration up to 0.01M, at which concentration the uptake was about 1.4 $\mu\text{M}/\text{mg}$ planarian. About 90% of the radioactivity was extracted by cold 10% TCA, and the remainder

of the activity was about equally divided between ethanol and acetone extractions, and the TCA-insoluble fraction. Seven days after removal from the external phosphate, only about 50% of the radioactivity was extracted by cold TCA, 20 to 25% by the organic solvents, and the remainder was in the residus.

Of all the compounds tested, PO_4^{3-} was taken up the least, as shown in Fig. 10 which summarizes the total uptake in 2 days of each of the compounds investigated when supplied at a concentration of 0.01M.

F. Transfer of Radioactivity by Cannibalism

When donor planarians, labelled with either adenine- ^3H or propionate- $2\text{-}^{14}\text{C}$ were cannibalized by D. tigrina, a significant proportion of the ingested radioactivity was incorporated into the cannibal and retained for long periods (Table II). In the case of propionate, the radioactivity was distributed in a similar manner in the recipient as it was in the donor, i.e., about 20% was extracted by 80% ethanol, 20% by 30% ethanol, and the remainder was ethanol-insoluble. These proportions did not change markedly with time after ingestion, nor did they appear to differ depending upon the portion (head, midsection, or tail) of the victim which had been cannibalized. In the case of cannibals eating adenine-labelled planarians, 40% of the radioactivity was TCA-insoluble, as compared to 5 or 10% in the donors. Again, this fraction did not appear to change markedly with time.

In other experiments carried out at the same time with propionate-fed worms, the rate of disappearance of label from worms transsected and allowed to regenerate did not differ markedly from that of planarians removed from the radioactive solution, that is, the sum of the halves approximated the whole.

G. Radioautographic Studies

Radioautographic studies of planarians grown in adenine and propionate showed, in general, a rather uniform distribution of label. There were essentially no silver grains above background on the surface of the worms, indicative that the activity found by the chemical analyses was not merely adsorbed on the planarian.

Though the concentration of silver grains, representing radioactivity, was relatively uniform, the highest concentration was over the cells of the digestive system and the lowest was over the nervous system. (Figs. 11 through 13).

DISCUSSION

At the time we started our studies of the radioactive uptake of various organic compounds by planarians, we were aware only of the study by Flickinger to establish their axial metabolic gradient when the flatworm was exposed to glycine or to $^{14}\text{CO}_2$ for 2 h.³ Hammen and Lum, also in short-time experiments, showed the rapid conversion of propionate-2- ^{14}C into alpha-ketoglutaric, succinic, fumaric, malic, citric, isocitric, lactic, and mesoxalic acids, and the formation of aspartic and glutamic acids and threonine from $^{14}\text{CO}_2$.⁴ More recently, Best, Rosenvold, Souders, and Wade have investigated the long-term uptake of leucine- ^{14}C and uracil- ^{14}C by feeding appropriately labelled yeast to the planarians over a period of several weeks to several months.⁵ Although each of these investigations was able to demonstrate incorporation of radioactivity into the planarians, in all cases the amount of radioactivity incorporated was very low. For example, the specific activity of the leucine- ^{14}C fed to the planarians by Best et al. was 5.5 $\mu\text{Ci}/\text{mM}$, and it resulted in planarians labelled to the extent of 2.0 $\mu\text{Ci}/\text{mM}$ after 7 weeks of feeding. Best et al. report that an individual planarian (size not stated, but estimated to be about 1.5 mg from the protein analysis given) contains 0.02 μM of leucine. On this basis, each planarian contained 88 dpm in the protein fraction. In other experiments, Best et al. attempted to label planarians by soaking in amino acid solutions containing 0.02 μCi leucine- $^{14}\text{C}/\text{ml}$ of unstated specific activity. Under these conditions, it is not surprising that uptake could not be readily demonstrated.

In our experiments, using L-leucine with a specific activity of 1 mCi/mM and 0.01M concentration, the activity found in the TCA-insoluble fraction was over 5000 dpm/100 μg planarian protein after 2 days of soaking. Using 100 μM and 100 μCi of leucine in 10 ml of water, and 4 worms/ml, 40 planarians could

be labelled to this extent. However, L-leucine is among the least efficient compounds that we tested for labelling planarians. The fatty acids, in particular, were much more effective as precursors. For example, when valerate is supplied at an external concentration of 0.01M or 10 μ M/ml, within two days the activity inside the planarian is equivalent to that of a 0.1M valerate solution. In other experiments, not summarized in this report, planarians were maintained for periods up to 4 weeks in acetate or propionate solutions (0.01M), and for periods up to 15 days in 0.003M adenine solutions. In these long-term experiments, the total radioactivity tended to saturate or plateau after approximately 4 to 7 days. A practical difficulty with long-term experiments, particularly those utilizing amino acids, was bacterial contamination of the solutions. This became noticeable after 2 or 3 days. To minimize this contamination, we generally transferred the planarians to fresh radioactive solutions every 2 or 3 days. In addition, in several experiments, the addition of 6 mg penicillin G and 6 mg of streptomycin-dihydrostreptomycin per 100 ml of solution reduced but did not eliminate bacterial growth.

Best et al. have expressed concern about the radiation dose to which planarians are exposed in soaking experiments. We believe it is more proper to take the average energy of the ^{14}C beta (0.055 MeV) rather than the maximum energy when calculating the radiation dosage received. On this basis, a solution containing 10 $\mu\text{Ci/ml}$ would result in a dosage of about 50 rep over a 2-day period. When the calculated uptake is 10 $\text{m}\mu\text{M}/100 \mu\text{g}$ planarian protein, the "concentration" of radioactivity inside the planarians equals the external concentration. If ^3H -labelled compounds are used, generally higher radioactive concentrations are employed to compensate for the decreased counting efficiency encountered. However, the average energy from tritium is about one-tenth that

from carbon, so it is unlikely that excessive radiation is received by the planarians in a typical experiment.

In terms of our search to find a method by which a large proportion of the radioactivity could be incorporated into high-molecular-weight compounds, cytosine appears to be the compound most efficacious. After several days in a cytosine solution and then seven days out, about 75% of the radioactivity of planarians was not extracted by cold TCA. More work should be done with cytosine- ^{14}C so that the compounds into which cytosine is incorporated can be more readily studied by chromatographic and radioautographic techniques.

Footnotes and References

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Table I. Comparison of uptake and retention of nucleic acid precursors into cold 10% TCA-insoluble material. Results are expressed in terms of μM equivalent of radioactivity found in the 10% TCA-insoluble fraction/100 μg of planarian protein, and the percentage of the total uptake which this represents. Total uptake data are given in Fig. 3. Adenine and cytosine data are from several experiments which have been combined.

Time in radio- active solution	<u>Adenine-3-^{14}C</u>		<u>Cytosine-3H</u>		<u>Uracil-2-^{14}C</u>	
	<u>TCA- insol.</u>	<u>% total in TCA- insol.</u>	<u>TCA- insol.</u>	<u>% total in TCA- insol.</u>	<u>TCA- insol.</u>	<u>% total in TCA- insol.</u>
3 h			0.12	28	0.002	2
1 day	0.1	4	0.25	33	0.03	6
2 days	0.16	4	0.37	59	0.03	5
3 days			0.38	62	0.07	10
3 days in + 1 day out			0.32	76		
2 days in + 3 days out	0.66	14	0.40	69		
+ 7 days out	0.64	19	0.32	82		
+ 13 days out	0.87	32	0.29	83		
+ 28 days out	0.49	31	0.10	83		
	<u>Adenosine-3H</u>		<u>Cytidine-3H</u>		<u>Uridine-2-^{14}C</u>	
3 h	0.006	17	0.005	16	0.009	15
1 day	0.03	25	0.025	18	0.027	23
2 days	0.05	45	0.09	36	0.07	22
3 days	0.06	43	0.04	29	0.08	26
3 days in + 1 day out	0.05	48	0.05	29	0.12	36

Table II. Radioactivity transferred by feeding radioactive planarians.

Days after feeding	<u>"Propionate" planarians</u>		<u>"Adenine" planarians</u>
	Expt. 1 dpm/four cannibals	Expt. 2 dpm/four cannibals	Expt. 1 dpm/four cannibals
1	71,000		256,000
2		43,000	
3	44,000		203,000
4		32,000	
7		20,000	
8	39,000		205,000
15	40,000		86,000

WUB-12404

Fig. 1. Effects of concentration and time on the total uptake of adenine-3-¹⁴C by *D. tigrina*. The concentrations of adenine in Expt. 1 were 0.6, 1.1, 3.5, 4.0, 10.9 and 11.4 μM/ml; in Expt. 2 concentrations were 2.4, 8.3 and 10.0 μM/ml. The radioactive concentrations of adenine-¹⁴C used was between 5 and 15 μCi/ml, and that of adenine-³H was 50 μCi/ml. Data were obtained from one planarian for each point in Expt. 1, and two planarians for each point in Expt. 2. Results are expressed in terms of mM of compound incorporated per 100 μg of planarian protein.

MUB-12405

Fig. 2. Loss of radioactivity after removal of *D. tigrina* from an adenine solution.

MUB-12406

Fig. 3. Fifteen *D. dorotocephala* were placed in a 10 ml beaker containing 10.4 to 10.9 μ M of the radioactive compound dissolved in 4 ml of spring water and the pH was adjusted to 7.0 with NaOH. Approximately 30 μ Ci of a ¹⁴C-labelled compound or 200 μ Ci of a ³H-labelled compound was used. Two planarians were sampled at each time interval. The uptake of cytidine and adenosine was nearly equivalent, and only one line has been placed on the graph for these two compounds.

MUB-12407

Fig. 4. The uptake and retention of adenine and cytosine by D. dorotocephala in terms of total uptake and uptake into the TCA-insoluble (nucleic acid) fraction.

MUB-12408

Fig. 5. The uptake and retention of glycine-2- ^{14}C , DL-alanine-2- ^{14}C , L-valine-4- ^{14}C , L-serine-3- ^{14}C , and L-leucine-3- ^{14}C into 10% TCA-soluble material by D. tigrina. The planarians were in the radioactive amino acid solution ($10\text{ }\mu\text{M/ml}$; sp. act. approximately $0.5\text{ }\mu\text{Ci}/\mu\text{M}$) for periods up to 2 days, after which they were placed in water for periods up to 5 days. Data for the TCA-insoluble fraction are given in Fig. 6.

MUB-12409

Fig. 6. The uptake and retention of amino acids in the 10% TCA-insoluble material by D. tigrina. Data for TCA-soluble fraction are given in Fig. 5.

Fig. 7. The uptake of glycine-2-¹⁴C by *D. tigrina* as a function of concentration and time. In this experiment, the ethanol fractionation procedure was used, and the fraction extracted is indicated by the dashed lines.

Fig. 8. Rate of uptake and retention of five labelled fatty acids from 0.01M solutions, pH7, by *D. tigrina*. The substrates were all alpha-labelled (except for formate), and 20 to 50 μCi of each was used in each 4 ml of solution. After 2 days in the radioactive solution, the planarians were removed to fresh bowls of water.

ZN-5981

Fig. 9. Radioautograph of 10% TCA-extractable material from planarians soaked in 0.01M valerate for 3 days. The TCA was first removed by continuous liquid-liquid extraction, and the aqueous phase was placed on Whatman No. 1 filter paper and chromatographed in phenol-water and then butanol-propionic acid water. Identified compounds include aspartic acid, glutamic acid, glutamine, and alanine. Analysis of the radioactive origin by hydrolysis in HCl and rechromatography yielded largely glucose.

Fig. 10. Summary of total uptake of phosphate, sugars, amino acids, nucleic acid bases, and fatty acids from 0.01M solutions in two days by planarians. Uptake is expressed in terms of mM equivalent of radioactivity/100 μg planarian protein.

ZN-5983

Fig. 11. Low-power photomicrograph of a longitudinal section of the anterior end of a D. tigrina. The relatively unstained area is neural tissue, and the digestive tract is the tree-like area in the center.

ZN-5984

Fig. 12. Radioautograph of the digestive tract from a planarian maintained in propionate- ^{14}C for 3 days.

ZN-5982

Fig. 13. Radioautograph of the neural tissue of the same animal as in Fig. 12. Note the relative absence of silver grains.

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