PROTEIN AND AMINO ACID TURNOVER DURING DIFFERENTIATION IN THE SLIME MOLD

II. INCORPORATION OF [^{35}S]METHIONINE INTO THE AMINO ACID POOL AND INTO PROTEIN

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(Received January 4th, 1960)

SUMMARY

In the slime mold, the size of the endogenous "free" methionine pool is a function only of the stage of development, and is uninfluenced by exogenous methionine. However, exogenous [^{35}S]methionine can exchange with endogenous methionine, and the extent of this exchange (*i.e.*, the specific radioactivity of pool methionine) is a linear function of the exogenous [^{35}S]methionine concentration. The manner in which cellular pool methionine molecules are utilized for protein synthesis is complex; a possible interpretation is suggested in DISCUSSION.

Active protein turnover occurs during differentiation in the slime mold. Methionine protein is replaced by endogenous pool [^{35}S]methionine at the rate of about 7 %/h at preculmination. The rate of protein synthesis appears to decrease slightly during development. Subsequent to a "pulse" of [^{35}S]methionine, the specific radioactivity of various protein classes can differ as much as three-fold. The relative rates of [^{35}S]-methionine incorporation into ethanol soluble as compared to ethanol insoluble protein changes during the course of differentiation.

INTRODUCTION

The results of previous studies on the activity of various enzyme proteins during development necessitated an investigation of the general characteristics of the amino acid pool and of protein synthesis in the slime mold. Experiments designed for this purpose have revealed some unusual properties of the endogenous amino acid pool and of protein synthesis in this differentiating system.

It will be shown that active protein turnover occurs during multicellular development in the starving slime mold. The present study demonstrates that the amount of endogenous methionine per cell is a function of the stage of differentiation. At no stage of development can increasing concentrations of exogenous methionine alter the *size* of the endogenous methionine pool, although increasing concentrations do enhance the *rate of exchange* of the pool with the exogenous (labeled) methionine. Evidence

will be presented suggesting that these exchangeable methionine molecules extracted by cold trichloracetic acid (TCA), may be qualitatively different with respect to protein synthesis.

MATERIALS AND METHODS

L-[^{35}S]*methionine* was obtained from Abbott Laboratories. The [^{14}C]amino acid mixture was prepared from an algae hydrolysate by Dr. R. HENDLER.

Bonner's salt solution contains in 1 l: NaCl, 0.6 g; KCl, 0.75 g; $CaCl_2$, 0.30 g (see ref. 1).

Dictyostelium discoideum: the myxamoebae were grown with *E. coli* strain ML 304d on a rich medium, washed and plated on purified agar[2] prior to studies on [^{35}S]methionine incorporation.

Methionine was assayed quantitatively according to the procedure described previously[3].

Protein was measured with the biuret method[4] or the absorption at 280 mμ and 260 mμ (see ref. 5).

Protein purification, prior to specific radioactivity determinations, was carried out by the following procedure: (a) precipitation of the total cellular protein by the addition of an equal volume of 6 % TCA, followed by centrifugation and the addition of 6 % TCA to the precipitate, and by boiling at 100° for 15 min; (b) dissolving the protein in 0.5 *N* NaOH, centrifuging and discarding any insoluble material; (c) reprecipitation with 12 % TCA and washing once with 6 % TCA; (d) dissolving the protein in 0.5 *N* NaOH, for quantitative protein and radioactivity determinations. The latter were corrected for self-absorption due to the presence of the alkali. Further purification did not alter the specific radioactivity of the protein samples. When ethanol soluble protein was to be purified (for comparison in specific radioactivity to the ethanol insoluble protein from the same cells), the extraction with 2.0 ml of 75 % ethanol at 45° was carried out immediately following precipitation of the total cellular protein by cold 6 % TCA. After reprecipitation from the ethanol by 12 % TCA, the ethanol soluble protein was carried through steps b–d. Before ethanol insoluble protein was carried through these steps, it was boiled 15 min at 100° in 6 % TCA.

Methionine isolation from protein was carried out as follows: the sample was hydrolysed in 6 *N* HCl in a sealed tube for 4 h at 105° (see ref. 6) . After removing excess HCl by repeated evaporation with heat, the hydrolysate was chromotographed on Whatman No. 1 paper using a solvent (*tert*-butanol–methyl-ethyl ketone–water–diethyl amine (40:40:20:4)) which separates methionine completely from the other major radioactive component found to be present. The latter is not cysteine, and is presumably an oxidation product of methionine[6]. The methionine area (R_F, 0.5–0.6) was eluted for quantitative microbiological assay and radioactivity measurement.

Methionine isolation from solution: A TCA cell extract containing free methionine was passed through a Dowex 50 H^+ (200–400 mesh X 12 cross linked) resin contained in a small tube (1 cm diameter × 10 cm length). The resin (about 1 ml) was washed with 10 ml of 0.01 *M* HCl, followed by 5 ml of water, and the amino acids were eluted quantitatively with 10 ml of 1 *M* NH_4OH. The sample was evaporated to dryness and dissolved in water for further analysis.

RESULTS

Properties of the cellular methionine pool

Kinetics of incorporation of [^{35}S]methionine: The labeling of the amino acid pool and of total cellular protein was followed as a function of time. Cells were grown and plated on washed agar in the usual manner. At late aggregation, 5.0 ml of Bonner's salt solution containing 0.3 μmoles of [^{35}S]methionine ($1 \cdot 10^6$ counts/min/μmole) were added to each of 12 plates. At 15, 60, 90 and 120 min thereafter the cells from groups of three plates were harvested, washed once with Bonner's salt solution containing unlabeled methionine and once with the salt solution alone*. Each sample was frozen until the completion of the experiment, at which time three types of analysis were made on each sample: (a) The size of the methionine pool (total μmoles methionine

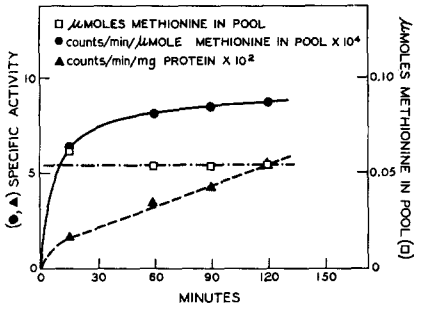

Fig. 1. The labeling of the amino acid pool and of total cellular protein as a function of time. The specific radioactivity of exogenous [^{35}S]methionine was $1 \cdot 10^6$ counts/min/μmole See text for details.

per three plates in the cold TCA extract[3]). (b) The specific radioactivity of the amino acid pool methionine and (c) the specific radioactivity of the total cellular protein. The data are given in Fig. 1.

The size of the amino acid pool did not change during the course of this experiment (although during the longer course of differentiation it decreases strikingly in size[3]). The greatest increase in specific radioactivity of the amino acid pool occurs in the first 15 min, and the maximum specific radioactivity of pool methionine attained at equilibrium is about $8 \cdot 10^4$ counts/min/μmole, which is an order of magnitude lower than that of the exogenous methionine ($1 \cdot 10^6$ counts/min/μmole). As will be seen below, only at preculmination does the pool methionine approach the external methionine in specific radioactivity. A kinetic picture similar to the one summarized by Fig. 1 is also observed at other stages of development, as shown in Table I.

Incorporation at different [^{35}S]methionine concentrations: Experiments were carried out in which the incorporation of [S^{35}]methionine into the amino acid pool and into protein was studied as a function of the concentration of externally supplied [^{35}S]methionine of constant specific radioactivity. In the experiment summarized by Fig. 2, carried out at the amoeba stage, the cells were harvested at 45 min, at which

* Further washing does not further decrease the specific activity of the methionine in the amino acid pool, demonstrating the absence of contaminating exogenous methionine which has, relatively, a much higher specific activity (see below).

time the free amino acid pool had attained its maximal specific radioactivity for each external methionine concentration. A surprising result is the fact that, in contrast to many other microorganisms, the size of the cellular methionine pool is independent of exogenous methionine concentration over the range investigated (0.04–0.32 μmoles/ml). Nevertheless, the specific radioactivity (*i.e.*, the extent of exchange between exogenous and endogenous methionine) of the methionine pool increases linearly with increasing external methionine concentration, although at this stage of development it never attains the specific radioactivity of the exogenous methionine. The curve for protein specific activity does not increase linearly, but tends to fall off at higher exogenous methionine concentrations. This phenomenon will be discussed below in the section dealing with protein synthesis.

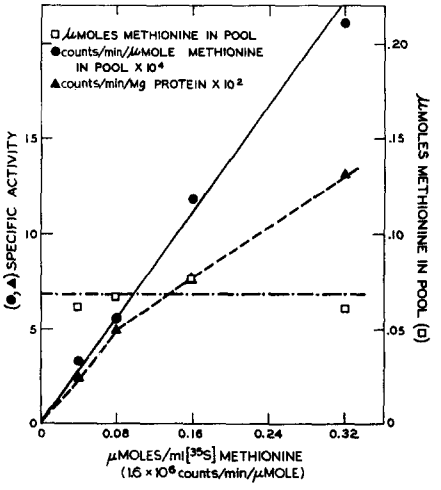

Fig. 2. Incorporation of [^{35}S]methionine into the amino acid pool and into protein as a function of the concentration of exogenous [^{35}S]methionine at constant specific radioactivity. See text for details.

Data are presented in Table I confirming and extending the two main results summarized by Figs. 1 and 2: (a) For a given exogenous [^{35}S]methionine concentration, the maximum specific radioactivity of the methionine pool is attained in about 20 min, and this value is less than the exogenous methionine specific radioactivity; (b) Increasing the concentration of exogenous [^{35}S]methionine does not "expand"[7] the methionine pool, but does increase its specific radioactivity. Table I also illustrates the fact that the size of the amino acid pool as well as the exchangeability of its amino acids is dependent upon the stage of differentiation.

At the three stages of development indicated in Table I, cells were exposed for 15 or 45 min to two different exogenous [^{35}S]methionine concentrations. At 15 and 45 min, for each methionine concentration, cells were harvested and washed in the manner previously described. For each sample, the absolute amount of methionine in a (comparable) cell aliquot was determined, as was the specific radioactivity of the amino acid pool and total cellular protein. It can be seen that the size of the methionine pool drops during differentiation, and that it is never increased by a ten fold increase in exogenous methionine concentration. For each stage of development the maximal

specific radioactivity which is attained in 15 min is a linear function of exogenous methionine concentration, and therefore increases 10 fold from the lowest to the highest exogenous [^{35}S]methionine concentration (see also Fig. 2). Note that, for a given exogenous methionine concentration, the specific radioactivity (exchangeability) of the endogenous methionine pool is maximal at the slug stage. Other experiments demonstrate that maximal exchangeability actually occurs at preculmination.

TABLE I

SIZE AND SPECIFIC RADIOACTIVITY OF THE CELLULAR METHIONINE POOL AS A FUNCTION OF (a) THE STAGE OF DIFFERENTIATION AND (b) EXOGENOUS [^{35}S] METHIONINE CONCENTRATION

Stage	*Exogenous [^{35}S]methionine μmoles/ml*	*Time exposed to [^{35}S]methionine (min)*	*Total μmoles methionine in cells*	*Counts/min/μmole methionine in cells × 10^6*
Amoeba	0.04	15	0.093	0.0156
	0.04	45	0.105	0.0130
Amoeba	0.40	15	0.114	0.123
	0.40	45	0.096	0.144
Slug	0.04	15	0.021	0.125
	0.04	45	0.015	0.116
Slug	0.40	15	0.017	0.913
	0.40	45	0.016	1.210
Fruit	0.04	15	0.022	0.033
	0.04	45	0.021	0.056
Fruit	0.40	15	0.020	0.415
	0.40	45	0.020	0.487

In order to obtain some preliminary information concerning the mechanism of exchange between the amino acid pool and exogenous methionine, the effect of 2,4-dinitrophenol was studied. Table II demonstrates that the exchange reaction (as well as protein synthesis) is inhibited about 50% at $4 \cdot 10^{-4}$ *M* 2,4-dinitrophenol, suggesting an energy-dependent process.

TABLE II

EFFECT OF 2,4-DNP ON POOL EXCHANGE AND PROTEIN SYNTHESIS AT PRECULMINATION

Concn. 2,4-DNP	*% inhibition*	
	Exchange	*Protein*
$2 \cdot 10^{-4}$	34	32
$4 \cdot 10^{-4}$	55	47

Having thus established certain properties of the endogenous methionine pool of the slime mold, it is now possible to examine the characteristics of protein synthesis from this amino acid pool during differentiation.

Protein synthesis from the cellular methionine pool

Preliminary to studies on the rate of protein synthesis, it was necessary to establish that, at any stage of development, the specific radioactivity of total cellular

protein truly reflects the rate of replacement of protein methionine, and not, for example, the relative abundance of methionine-rich proteins. Therefore, the specific radioactivity of protein labeled at various stages of development was compared with the specific radioactivity of the methionine isolated from this protein. At the amoeba, slug, preculmination and fruit stages, cells were exposed to [^{35}S]methionine for 3 h in Bonner's salt solution. Incorporation of [^{35}]Smethionine into protein is linear after the first 15 min (Fig. 1). The total cellular protein was then isolated, purified, and its specific radioactivity determined. These values are represented by the solid circles in Fig. 3. An aliquot of each protein sample was then hydrolyzed and the specific radioactivity of the isolated methionine determined. It can be seen that the ratio of these two specific radioactivities is constant, in spite of the fact that at preculmination the values are four-fold those at the amoeba stage. We may conclude that at all stages of development, on the average, protein specific radioactivity truly reflects methionine specific radioactivity, and that the latter can be obtained from the former by applying a factor of 12.5 $\left(= \frac{\text{methione specific radioactivity}}{\text{protein specific radioactivity}}\right)$

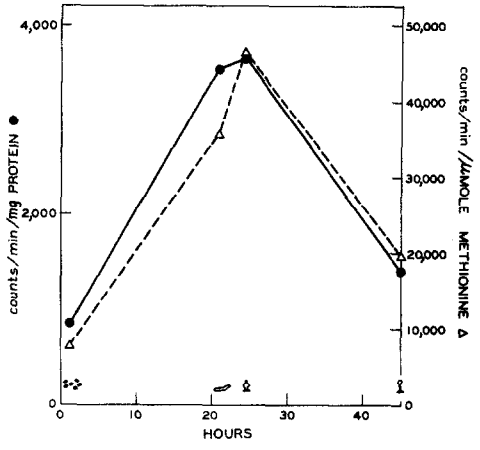

Fig. 3. The relationship of protein specific radioactivity to protein-methionine specific radioactivity, following a 3-h exposure to [^{35}S]methionine at 4 stages of differentiation.

It would appear from these data that the rate of protein synthesis increased almost one order of magnitude during early differentiation. We shall see below that, instead, the extent of labeling the amino acid pool increased, while the rate of protein synthesis from this pool if anything decreased.

Incorporation into protein at different [^{35}S]*methionine concentrations*: Although cellular pool methionine specific radioactivity is a linear function of exogenous [^{35}S] methionine concentration, this is not true of protein specific radioactivity. The data of Fig. 2 show the tendency of the protein curve to fall off from linearity as the exogenous [^{35}S]methionine concentration increases. Table III summarizes two other experiments in which the protein specific radioactivity failed to increase in parallel with the pool specific radioactivity, in response to a tenfold rise in exogenous [^{35}S]-methionine concentration. Thus the apparent rate of protein synthesis from the amino

acid pool (% incorporation of [^{35}S]methionine—last column) decreases as the specific radioactivity of the amino acid pool increases (see calculations below).

TABLE III

INCORPORATION OF [^{35}S]METHIONINE INTO PROTEIN

Expt.	*Stage*	*Exogenous [^{35}S]methionine μmoles/ml*	*Average counts/min/μmole pool methionine/30 min × 10^6*	*Counts/min/mg protein Δ/30 min**	*%** incorporation*
I	Amoebae	0.04	0.018	134	9.2
		0.40	0.11	660	7.6
	Slug	0.04	0.24	1,144	5.9
		0.40	2.10	6,360	3.7
II	Amoebae	0.04	0.014	127	11.1
		0.40	0.133	840	7.9
	Slug	0.04	0.120	1,282	14.4
		0.40	1.061	5,040	6.2

* 45 min value minus 15 min value.

** % incorporation $= \frac{\Delta \text{ protein specific act.} \times 12.5 \times 100}{\text{average pool methionine specific act.}}$

Calculation of the rate of protein synthesis: The formula used for calculating % incorporation is: % incorporation $= \frac{\Delta \text{ protein specific act.} \times 12.5}{\text{average pool methionine specific act}} \times 100$.

It is of course necessary to calculate the % incorporation of methionine into protein during a time period in which the pool methionine specific radioactivity is constant. Thus after optimal pool methionine specific radioactivity is attained, two values, at 15 and 45 min, are determined and averaged. Over this same time period (30 min) the increase in protein specific radioactivity is determined.

For example, in the case of the amoebae at 0.04 μmoles methionine/ml (Table III Expt. II), the increase in protein specific radioactivity, 127 counts/min, is multiplied by 12.5, (which is the ratio of protein methionine specific radioact./protein specific radioact.) thus converting it to counts/min/μmole methionine (see relationship of protein specific radioactivity to methionine specific radioactivity, Fig. 3). This value, 1,587, is divided by the average specific radioactivity of the amino acid pool over the 30-min period ($0.014 \cdot 10^6$ counts/min/μmole), giving the percent incorporation per 30 min period as 11.1 %. At the high exogenous [^{35}S]methionine concentration the rate of incorporation has decreased to 7.9 %.

There are two general explanations which could account for the (apparent or genuine) decrease in rate of protein synthesis at higher pool specific radioactivities: (a) High exogenous methionine concentrations inhibit protein synthesis. (b) The pool methionine molecules are not equivalent with respect to protein synthesis: *on the average*, the methionine molecules in the amino acid pool labeled at low pool specific radioactivities became incorporated into proteins attaining a relatively high specific radioactivity; pool methionine molecules labeled at high pool specific radioactivity become incorporated into proteins attaining a relatively lower specific radioactivity.

Effect of high exogenous methionine on protein synthesis: In order to test directly whether a high exogenous methionine concentration inhibits protein synthesis, the following experiment was carried out:

Cells at late aggregation were incubated in Bonner's salt solution for 7 min in the absence (A) and presence (B) of a mixture of [^{14}C]amino acids to label the amino acid pool. Each cell suspension was quickly centrifuged, and the unlabeled cells suspended in a salt–[^{14}C]amino acid mixture. Half of these cells were frozen immediately, and half were frozen after an additional 9-min incubation. The cells previously labeled (B) were divided and incubated in the absence (B1) and presence (B2) of unlabeled methionine (0.3 μmoles/ml). In both these cases, samples were again frozen at zero and 9 min. As can be seen from Table IV experiment A, active incorporation of exogenous [^{14}C]amino acids was occurring during the 9-min incubation period. The 0 min sample of Expt. A could not be frozen quickly enough to prevent all incorporation. Expts. B1 and B2 demonstrate that protein synthesis from [^{14}C]amino acids within the cells is not inhibited by a high concentration of unlabeled exogenous L-methionine.

TABLE IV

EFFECT OF EXOGENOUS METHIONINE ON PROTEIN SYNTHESIS

Expt.	*Additions*		*Counts/min/mg protein*		
	7 min preincubation	*9 min incubation*	*0 min*	*9 min*	*Δ*
A	None	[^{14}C]amino acids	272	625	353
B1	[^{14}C]amino acids	None	737	1,180	443
B2	[^{14}C]amino acids	Unlabeled methionine	777	1,187	410

We are thus left with the alternative general explanation that, at higher pool specific radioactivities (compared to lower pool specific radioactivities), relatively more labeled methionine molecules are incorporated into proteins of low specific radioactivity. We have in fact found clear heterogeneity in the specific radioactivities of various arbitrary classes of slime mold protein following a pulse of [^{35}S]methionine.

Heterogeneity in specific radioactivities of different protein classes: We have seen previously (Fig. 1) that incorporation of [^{35}S]methionine into total cellular protein is linear after the first 15 min. Specific radioactivities of ethanol soluble and ethanol insoluble protein were also followed as a function of time of exposure to exogenous [^{35}S]methionine. This experiment was carried out at preculmination, since the difference in the rate of incorporation of [^{35}S]methionine into these two classes of proteins is usually greatest at this stage of development (Fig. 4).

In this experiment, there is about a three-fold difference in the specific radioactivity of these two classes of proteins. The extent of this difference varies from experiment to experiment; the variables involved are not yet understood. Table V gives the ratio of specific radioactivities of these two protein fractions as a function of the stage of differentiation. At each of the stages indicated, the cells were exposed to [^{35}S] methionine (0.2 μmoles/ml) for 1 h, harvested, and the two types of protein purified for specific radioactivity determinations. Thus the specific radioactivity of

ethanol insoluble protein is higher than that of ethanol soluble protein at all stages of development, and this difference becomes maximal at preculmination.

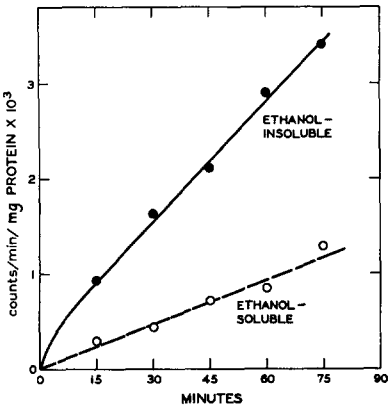

Fig. 4. The specific radioactivity of ethanol soluble and ethanol insoluble protein at preculmination as a function of time.

TABLE V

SPECIFIC RADIOACTIVITIES OF TWO CLASSES OF PROTEINS AS A FUNCTION OF THE STAGE OF DEVELOPMENT

Stage	*Specific radioactivity: ethanol insoluble / ethanol soluble (counts/min/mg protein)*
Amoeba	1.56
Aggregation	2.11
Early slug	2.27
Slug	2.70
Preculmination	4.15
Fruit	2.82

These changes in ratio observed as a function of differentiation may be due either to (a) differential rates of synthesis of the two classes of protein, or (b) differential rates of utilization of methionine rich (or poor) protein. Both phenomena could of course be involved.

In order to observe the specific radioactivities of many more classes of protein following a pulse of [^{35}S]methionine, DEAE column chromatography was used. The various protein classes resolved by this procedure showed heterogeneity in specific radioactivities, with maximal differences of about three-fold[8]. It is clear from these experiments that significant variations in protein specific radioactivities exist.

The rate of protein synthesis at different stages of development: A summary of calculated rates of methionine incorporation at various stages of development and at various external methionine concentration is presented in Table VI.

At any particular stage of development the rate of protein synthesis appears to decrease at relatively higher exogenous methionine concentrations (Table III). However, it can be seen from Table VI that comparing what seem to be similar stages in different experiments can give quite variable absolute rates of synthesis.

TABLE VI

% INCORPORATION PER 30 min INTO PROTEIN

Experiment	*I*	*II*	*III*	*IV*	*V*	*VI*
μmoles [^{35}S]methionine/ml	*0.04*	*0.04*	*0.10*	*0.20*	*0.40*	*0.40*
Amoebae	9.2	11.1			7.6	7.9
Blob			4.0	4.5		
Slug	5.9	14.4			3.7	6.2
Early preculmination			4.1	3.4		
Late preculmination				2.9		
Young fruit		3.9			4.4	
Old fruit			10.7	6.6		

The validity of the calculated rates of incorporation depend upon two assumptions: (a) That the TCA-soluble amino acid pool is the sole source of amino acid precursors for protein synthesis, and (b) that the pool methionine molecules labeled at all external [^{35}S] methionine concentrations are equivalent with respect to protein synthesis. That this second assumption may not be entirely correct appears evident from the results presented previously.

Since our data have shown that pool methionine molecules are heterogeneous with respect to their rate of exchange, it may not be possible to observe the "true" rate of their incorporation into protein except when complete exchange of external and pool methionine has occurred. This situation can at least be closely approximated at high exogenous methionine concentrations during preculmination, which is metabolically the most active stage of development. From Table VI it can be seen that, at the higher exogenous methionine concentrations during early and late preculmination (Expts. III and IV). the incorporation rate was between 2.9 and 4.1 %/30-min period, or about 7 %/h. In these cases, the specific activity of the amino acid pool was more than half that of the exogenous methionine. The data in this table, summarizing six experiments, suggest that the overall rate of protein synthesis decreases slightly during development and is minimal at preculmination. However, this decrease may be only apparent, if the relatively small fraction of pool methionine molecules which can be labeled in the early stages of development are not representative of the higher percentage of labeled molecules at preculmination.

DISCUSSION

In order to facilitate the discussion, a schematic description of some of the biochemical events occurring during differentiation in the slime mold is presented in Fig. 5. The first four items listed in the diagram are dealt with in other publications[2,3,9,10].

Because of the indicated increase in the rate of exchange of "free" amino acids in the cells, the pool and hence protein specific activities increase at the beginning and then decrease towards the end of differentiation. The increased rate of exchange of pool amino acids may in part be due to a decrease in the absolute size of the pool and may in part be a reflection of turnover: their rate of formation from protein breakdown and their rate of utilization. Since the rate of protein synthesis does not increase simultaneously with cellular amino acid turnover, it is quite possible that the latter increases from enhanced net protein breakdown coupled with intensive carbo-

hydrate synthesis from the resulting amino acids. The enhanced activity of these two metabolic processes at preculmination is well documented[3,9,10].

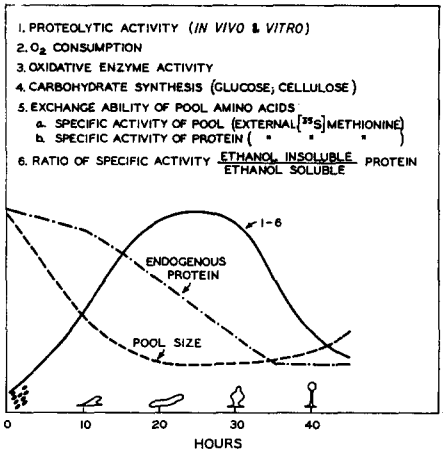

Fig. 5. A schematic representation of various biochemical changes occurring during differentiation in the slime mold. For further explanations, see text.

At preculmination, when the pool specific activity is almost as high as that of the exogenous [^{35}S]methionine, the rate of protein synthesis is about 7 %/h. This value can be compared to that of resting bacteria, 5 %/h[11], and that of animal cells in tissue culture, 1 %/h[12].

Judging from the behavior of methionine, and from total pool amino acid determinations[3], the size of the amino acid pool is a function only of the stage of development, uninfluenced by exogenous amino acids. This is significant in view of the requirement of starvation for the initiation of multicellular differentiation. Thus the slime mold cells have a barrier to concentrating at least one amino acid, although an exchange with existing molecules can occur. An analogy to this situation may be found in the exchange-diffusion of phosphate across cell walls[13]. Manometric studies indicate no oxidation (and hence no net uptake?) of exogenous methionine or serine[14], and determinations of changes in cell volume suggest that glycine and cysteine do accumulate inside the cell whereas arginine does not[15]. It is also clear that exogenous histidine accumulates in the cell[18].

In all probability, the cellular amino acid pool is the source of precursors for protein synthesis, particularly since the slime mold normally depends only on endogenous amino acids. Investigations in *E. coli* and in yeast demonstrate that externally-supplied amino acids must pass through a cellular pool prior to incorporation into protein[16,17*].

Assuming that only endogenous amino acids are used for protein synthesis, it would seem unlikely that increasing the exogenous methionine concentration tenfold could inhibit general protein synthesis by 50 %, particularly since the endogenous methionine pool is not expandable (Table I). Moreover, an experiment specifically

* If the rate of protein synthesis in the slime mold were calculated from the specific activity of exogenous methionine the rate would increase tenfold in going from the amoebae to the slug stage in Table III, Expt. I.

designed to test whether high exogenous methionine concentrations inhibit protein synthesis was negative (Table IV). In view of these considerations, it is felt that the decrease in the calculated rate of protein synthesis obtained when the pool specific radioactivity increases (as a result of development or as a result of increasing exogenous [^{35}S]methionine concentration at a given stage of development) is only apparent, and not real. The following tentative explanation is offered for this phenomenon: the fact that the methionine of the pool does not fully equilibrate with that of the external methionine indicates that, at different external methionine concentrations, labeled methionine equilibrates with different parts of a complex pool. In other words, some methionine molecules are more easily exchangeable than others[7]. (This lack of full equilibration with exogenous methionine is undoubtedly in part due to a high rate of formation of endogenous cold amino acids from protein catabolism.) As the specific radioactivity of the endogenous methionine pool increases, an increasing number of the methionine molecules which become labeled will be incorporated into proteins of low specific radioactivity, or will not become incorporated into protein at all. Hence an apparent decrease in the calculated rate of protein synthesis is observed. The heterogeneity found in specific radioactivity of various classes of protein could be due to (a) different methionine contents or (b) different rates of synthesis. Preliminary experiments support the latter interpretation[8].

If our speculation is valid, and pool amino acids have different fates with respect to protein synthesis, it is conceivable that changing the number or pattern of pool amino acids (*e.g.*, through starvation) could effect changes in the patterns of synthesized protein.

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