Discussion. Changes in donor cells must have been responsible for the observed differences in transplantation fraction since all recipient mice in these experiments were given the same treatment, namely, 900 rad irradiation. It is not known which characteristics of the cells determine transplantability or how these are affected by treatment with endotoxin, VLB, or radiation. If fragility is greater in the cells from treated mice one might suppose that its effects would be lost in the first transplantation, and that subsequent fractions would be more nearly normal. Changes in cell configuration and adhesiveness are also possibilities to be considered. Although the mechanisms responsible remain obscure, it is important to be aware of changes in transplantability which may be associated with treatment of the donor mouse.

Summary. Hemopoietic colony forming cells can be estimated from the assay of CFU, provided the transplantation fraction is known. The fraction for first transplantation cannot be determined by the present methods and has been assumed to be the same as the measurable fraction obtained from a second transplantation. Fractions of CFU recovered on third transplantation were found to be the same as on second, supporting this assumption. Transplantability was altered by treatment of donor mice with irradiation, endotoxin, and VLB. Thus, the loss in CFC between minutes after and 1 day after irradiation was less than the loss in CFU. Pretreatment of irradiated mice with endotoxin or VLB caused a greater reduction in the transplantation fraction than radiation alone. Thus, 1 day after irradiation, differences in CFC values between control and treated mice were even greater than the differences in CFU values previously reported. Clearly, changes in transplantability can be induced and this must be taken into account in the interpretation of effects of various treatments on hemopoietic colony forming cell populations.

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Received Feb. 5, 1968. P.S.E.B.M., 1968, Vol. 128.

Protein Synthesis in Frog Embryos and Frog Liver Preparations, and Its Inhibition by Embryo Components* (33016)

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In the course of frog development, the total protein content of the organism remains constant or decreases slightly during embryonic development and in the first days after hatching, and a net increase of total protein is not seen until some days after the larvae begin active feeding (1). The factors that control the pattern of protein turnover and net synthesis of protein during amphibian development have not been defined, and the detailed characteristics of the protein-synthesizing system in amphibians have

* This work was supported by Research Grant GM 09304 from N.I.H., USPHS.

not yet been fully defined. However, various activities associated with protein synthesis have been demonstrated in eggs or early embryonic stages of frogs. Thus, amino acid-activating activity was demonstrated in frog eggs (2,3) and in prehatching and hatched embryos of *Xenopus*, the "clawed toad" (4). Soluble RNA and DNA-like RNA are synthesized during early embryonic stages, and synthesis of ribosomal RNA begins at gastrulation (5,6). However, an increase in ribosomes is not observed until just before hatching, and there is also a significant shift of protein from particulate to soluble form at this time (7).

Incorporation of labeled amino acids into protein was observed when ovarian egg masses (8) or segments of gastrula stage frog embryos (9) were incubated in media containing labeled amino acids, or when labeled amino acids were injected into frog eggs (10). Similar studies with intact developing amphibian embryos are hindered by the apparently limited uptake of amino acids from the medium (11,12). Incorporation of labeled amino acids into protein has been reported with a cell-free preparation and with isolated nucleoli from frog ovarian eggs (2, 3), but analogous cell-free preparations have not been reported for developing frog embryos. However, observations on the appearance of specific antigens (13), on increases of enzyme activities (14), and on changes in isozyme patterns (15) suggest that new protein molecules are synthesized in developing frog embryos and larvae prior to the time when a net increase of total protein is seen. Degradation of preexisting protein, especially of yolk protein, can provide amino acid units for protein synthesis during the period of constant total protein content. Yolk protein content declines rapidly in the period just after hatching (16).

The present study was undertaken to define further the protein-synthesizing system in the frog, especially the capacities of the system during the developmental period shortly after hatching when net accumulation of protein begins. An active cell-free system for amino acid incorporation into protein was first obtained from adult frog liver. In contrast, analogous cell-free preparations from frog embryos or from larvae after hatching showed little incorporation of amino acids into protein, although rapid incorporation of amino acids by intact larvae was observed. Cell-free preparations from early developmental stages were found to contain a component that strongly inhibited amino acid incorporation by the cell-free system from adult frog liver, and the frog liver system was used to define some characteristics of this inhibitory substance.

Methods. Animals. Gravid female and mature male *Rana pipiens* (leopard frogs) were maintained at 3°C. Induced ovulation, fertilization, and subsequent maintenance of embryos and larvae were carried out at 21°C. The induced ovulation and fertilization followed the procedure of Rugh (17). The embryos were allowed to develop in a saline growth medium containing, per liter, 0.35 gm of NaCl, 0.005 gm of KCl, 0.01 gm of $CaCl_2$ and 0.004 gm of $NaHCO_3$; similar development and growth curves were obtained with a medium only 10% as concentrated. Larvae were fed parboiled lettuce, beginning 2 days after hatching. Embryo ages are defined in terms of Shumway stages (18), and the ages of older larvae are given in terms of days past attainment of Shumway Stage 25.

Cell-free preparations. Samples (5–10 gm) of freshly excised liver from mature female frogs were rinsed with and weighed in cold medium A, which contained 0.25 *M* sucrose, 0.05 *M* Tris buffer, pH 7.8, 0.05 *M* KCl, 0.01 *M* $MgCl_2$ and 0.003 *M* β-mercaptoethanol. Frog eggs, embryos and larvae were washed with water before collection in medium A. All subsequent steps were carried out at 0–2°C. A suspension of liver or embryonic material in 2 volumes (w/v) of medium A was homogenized with a Teflon–glass homogenizer to produce 90% cell breakage, except that only 50% cell breakage was obtained with embryos of stages near the time of hatching, despite a more prolonged homogenization. Subjection of liver preparations to the prolonged homogenization did not significantly alter their amino acid-incorporating capacity. The homo-

genate was centrifuged at 15,000*g* for 10 min in an anglehead rotor, and the resulting supernatant fluid was recentrifuged under the same conditions. Again the supernatant fluid was retained, diluted with medium A, and designated Extract (E). A portion of E was centrifuged at 110,000*g* for 90 min to yield a microsomal pellet and a postmicrosomal supernatant fluid (PMS). The microsomal pellet was gently rinsed with medium A and resuspended in medium A to yield the microsomal fraction (M). A "pH 5 fraction" was obtained from PMS by adjusting to pH 5.0 with acetate buffer, pH 4, recovering the resulting precipitate by centrifugation, and resuspending the precipitate in medium A. The supernatant fluid from the pH 5 precipitation was adjusted to pH 7.8 with NaOH and designated the soluble fraction (S). Preparations usually were stored at −15°C overnight before use; this procedure entailed only minor loss of amino acid-incorporating activity. The protein content of homogenates and fractions was determined by the micromethod of Lowry (19).

Amino acid incorporation with cell-free systems. The standard incubation mixture contained, in a total volume of 1 ml: 0.02 *M* Tris buffer, pH 7.8; 0.06 *M* KCl; 0.10 *M* sucrose; 0.005 *M* $MgCl_2$; 0.005 *M* β-mercaptoethanol; an energy generating system, consisting of 1 μmole of ATP, 1 μmole of phosphoenolpyruvate, and 10 μg of pyruvate kinase; 0.2 μmole of GTP; 0.65 mg of polyuridylic acid (Miles Laboratory); 0.5 mg of yeast soluble RNA (Nutritional Biochemicals or Schwarz BioResearch); 1 μC of uniformly labeled L-phenylalanine-^{14}C (Schwarz BioResearch, specific activity 250 mC/mmole); and tissue fractions, usually fraction M plus pH 5 fraction. The amount of tissue fractions added usually was that obtained from 0.16 gm of wet weight tissue; these amounts of fraction M and of pH 5 fraction from liver contained 1.5–2.5 mg and 1.0–2.0 mg of protein, respectively. Activity was proportional to the amount of fraction M plus pH 5 fraction, with the amounts of these fractions obtained from 0.03 to 0.24 gm of wet weight of liver. The tissue fractions were added last to the ice-cold incubation mixture, and the mixture was incubated at 30°C in a water bath with shaking. The incubation was ended, usually after 60 min, by adding 0.4-ml aliquots of the mixture to 2-ml aliquots of 10% trichloroacetic acid (TCA) containing 1.0% unlabeled D,L-phenylalanine. As controls, aliquots of the incubation mixture were added to the TCA at 0 min. The TCA suspensions were incubated at 85°C for 30 min, then cooled, and the residual precipitates were collected by centrifugation. The precipitates were washed twice by centrifugation with 2-ml aliquots of 5% TCA containing 0.5% D,L-phenylalanine and once with 5% TCA. The amount of label incorporated into the TCA precipitates was not significantly reduced by extraction of the precipitates with 1:4 ethanol:ether, and this extraction was therefore not routinely performed. The washed precipitates finally were dissolved in 1 ml of Hyamine, and radioactivity measurements were made in a liquid-scintillation counter with 50% efficiency. Unless otherwise indicated, the incorporation values reported were corrected for background and for counts in samples from controls in which the incubation had been terminated with TCA at 0 min.

TABLE I. Total Protein Content of the Developing Frog.[a]

Developmental stage	Protein (mg/embryo or larva) Mean[b]	±SD[c]
Stage 2 (fertilized egg)	0.99	.05
Stage 21 (hatched 1 day)	0.79	.06
Stage 25 (feeding 2 days)	0.79	.07
Stage 25 + 5 days	0.84	.08
Stage 25 + 10 days	1.09	.02
Stage 25 + 25 days	1.81	.37
Stage 25 + 35 days	3.04	.44
Stage 25 + 45 days	3.86	.68

[a] Pooled embryos or larvae of a designated developmental stage were homogenized in 0.02 *M* phosphate buffer, pH 7.4, and aliquots were assayed for protein content by the micromethod of Lowry (19).
[b] Mean of results from 3–8 experiments.
[c] Standard deviation.

Results. Protein content. Table I sum-

TABLE II. *In Vivo* Uptake and Incorporation of Amino Acids by Frog Larvae.[a]

Developmental stage	Radioactivity (cpm/larva) after 1 hour		2 hours	
	Protein	Non-protein[b]	Protein	Non-protein[b]
Stage 20	0	46	0	54
Stage 21	5	42	5	80
Stage 22	7	65	17	142
Stage 23	10	269	45	338
Stage 24	90	204	235	301
Stage 25	182	507	372	592
Stage 25 + 2 days	299	466	817	700
Stage 25 + 4 days	393	537	1,166	714

[a] Groups of 20 embryos of designated developmental stage were incubated for 1 or 2 hours in 40 ml of saline containing 0.035 μC L-phenylalanine-^{14}C (specific activity 250 mC/mmole) per ml. Embryos were quickly collected, rinsed and homogenized in cold acetone. Aliquots of homogenates were plated and the radioactivity determined in a gas-flow counter with 15% efficiency, as a measure of total uptake. Other aliquots were precipitated with 10% TCA–1% unlabeled phenylalanine, washed with TCA, extracted with acetone, plated and counted, as a measure of incorporation into protein. Data were corrected for background counts; self-absorption was negligible. Controls in which larvae were incubated for 15 sec gave counts for total uptake that were not significantly higher than background.

[b] Nonprotein = difference between total uptake counts and counts incorporated into protein.

marizes the total protein content of *Rana pipiens* embryos at various stages of development. The level of about 1.0 mg of protein in the fertilized egg was maintained in subsequent developmental stages prior to hatching (data not tabulated), which occurs at Stage 20, 6 days after fertilization. The lower protein content at Stage 21, 1 day after hatching, reflects largely the loss of jelly and embryonic membranes at hatching. Although the larvae began active feeding at Stage 23, 3 days after hatching, the protein content of Stage 25 larvae, 5 days after hatching, was still at the Stage 21 level; a significant increase of total protein was not noted until about Stage 25 plus 5 days.

Amino acid uptake and protein synthesis in vivo. Table II summarizes studies on uptake of labeled phenylalanine from growth medium and its incorporation into protein by frog larvae at hatching (Stage 20) and at subsequent developmental stages during 1- and 2-hour incubations. The "nonprotein" radioactivity may include various nonprotein metabolites produced from the labeled amino acid taken up, but nevertheless reflects the amount and concentration of the amino acid taken up during the incubation period. Amino acid uptake, and, therefore, the concentration of labeled precursor for protein synthesis was at a low level in Stage 20 larvae and increased gradually to high levels in Stage 23 and older larvae. Larvae up to Stage 23, when feeding began, showed only low levels of incorporation into protein, and markedly higher levels began with Stage 24 larvae. Higher uptake appeared to precede slightly the marked increase of incorporation into protein.

Protein synthesis by cell-free system from frog liver. As is shown in Table III for a typical fractionation of adult frog liver, a cell-free extract (E) actively incorporated L-phenylalanine-^{14}C into TCA- precipitable material in an appropriately supplemented *in vitro* incubation system. The major portion of this activity was recovered in the combination of the microsomal fraction (M) and the pH 5 fraction obtained from the extract. While activity levels varied in preparations from different batches of frogs, and varied with time of year, the incorporation seen with fraction M plus pH 5 fraction from typical winter frogs was usually 10–20 times that of the 0 time control. The characteristics of the incorporating system were not altered by these variations of activity levels.

The effects of various additions to or deletions from the standard incubation system are summarized in Table IV. Both fraction M and the pH 5 fraction were required to obtain appreciable activity, and the pH 5 fraction was the most limiting tissue fraction. Doubling the concentrations of other components in the reaction mixture either singly or in combinations produced only minor or negligible changes (not tabulated). Polyuridylic acid (poly U) was essential,

TABLE III. Incorporation of L-Phenylalanine-^{14}C into Protein by Cell-Free Fractions from Frog Liver and from Frog Larvae.

Source of preparation	Fractions used and mg of protein in fractions[b]	Incorporation (cpm) in 0 min control	Incorporation (cpm) after 60 min incubation[c]
Frog liver	E (14.3)	553	14,405
	M (2.0) + pH 5 (1.0)	534	9,875
	M (2.0) + pH 5 (1.0) + S (6.9)	612	11,860
Larvae, Stage 21	E (5.8)	491	522
	M (1.2) + pH 5 (0.9)	294	392
Larvae, Stage 25 + 6	M (0.9) + pH 5 (0.4)	621	778
Larvae, Stage 25 + 31	M (0.6) + pH 5 (0.5)	269	362

[a] Cell-free fractions were obtained from adult frog liver or from whole larvae as described under "Methods." The fractions were assayed in the standard *in vitro* incubation system described under "Methods" for incorporation of L-phenylalanine-^{14}C, and ^{14}C was counted in the TCA-precipitable material.

[b] The amounts [given in parentheses (mg)] of fractions used were obtained from the following amounts, wet weight, of source material: 0.16 gm of frog liver, or 0.32–0.40 gm of whole larvae.

[c] Incorporation not corrected for 0 min control values.

apparently as the specific messenger RNA for polyphenylalanine synthesis; polyadenylic acid could not substitute for poly U. Of the other added cofactors (data not tabulated), the energy generating system was essential for significant activity, and GTP was required for maximal activity. Neither yeast soluble RNA nor an analogous preparation from liver had significant effect, but inclusion of yeast soluble RNA was continued because of possible effects in projected studies with embryo preparations. Activity was maximal with 0.003 *M* to 0.008 *M* Mg^{2+}, and decreased markedly with Mg^{2+} levels below 0.002 *M*.

The time-course of incorporation (not tabulated) showed a rapid initial rate of incorporation, then a slowing to essentially maximal incorporation in 40 min. Of the total incorporation seen after the standard 60-min incubation, about 33% was obtained in 5 min, 52% in 10 min, 78% in 20 min, 88% in 30 min, and 95% in 40 min. This time-course appeared to reflect exhaustion or inactivation of some component(s) of the tissue fractions, since the addition of more fraction M plus pH 5 fraction after 30 min of incubation resulted in an increased amount of ^{14}C incorporation at 60 min, whereas supplementation with additional amounts of cofactors at 30 min did not increase the amount of incorporation at 60 min.

The results of inhibitor studies, also shown in Table IV, are those expected if the ^{14}C incorporation into TCA-precipitable material represented typical polypeptide synthesis. While puromycin caused complete inhibition, the structurally related aminonucleoside had no effect. The lack of inhibition by actinomycin D suggests that the process is not dependent upon concomitant RNA synthesis, while the inhibitory effect of ribonuclease indicates that the incorporation process involves one or more essential RNA components.

The *in vitro* protein-synthesizing system from frog liver was strongly inhibited by the soluble fraction from Stage 21 frog larvae (Table IV). The analogous fraction S from frog liver showed no such inhibitory power (Table III), and analogous fractions from chicken liver, frog muscle, and frog kidney also were not inhibitory (not tabulated).

The capacity of the cell-free liver preparations to incorporate amino acid into protein was not limited to poly U-stimulated phenylalanine incorporation. When incu-

TABLE IV. Effects of Additions and Deletions on the Phenylalanine Incorporating System from Frog Liver.[a]

Change from complete standard incubation	Relative activity	
	Mean[b]	±SD[c]
(Standard system)	(100)	
— M fraction	5	4
0.5 × M fraction	89	7
— pH 5 fraction	6	4
0.5 × pH 5 fraction	55	5
2 × pH 5 fraction	176	19
— Cofactors[d]	3	3
— Poly U	6	4
+ 500 μg of puromycin[e]	1	2
+ 500 μg of aminonucleoside[e]	96	2
+ 50 μg of actinomycin D[e]	97	2
+ 40 μg of RNAase[e]	2	3
+ .01 ml of Stage 21 S fraction	1	1

[a] The standard *in vitro* incubation system described under "Methods," containing M and pH 5 fractions from 0.16 gm of frog liver, was used as a base line of incorporating activity. Incorporation under each altered condition was compared to that of the standard incubation system, using aliquots of the same preparation of liver fractions.

[b] Mean of results from 3–6 experiments.

[c] Standard deviation.

[d] Cofactors include energy generating system, poly U, GTP, and yeast soluble RNA.

[e] Neutral solutions of puromycin-HCl, aminonucleoside, and protease-free ribonuclease (all from Nutritional Biochemicals) and actinomycin D (a gift of Merck, Sharp and Dohme).

bated with 2 μC of ^{14}C amino acid mixture in place of 1 μC of phenylalanine, the ^{14}C incorporation by fraction M plus pH 5 fraction in 60 min was 40% of that obtained with phenylalanine; the 0 time control incorporation was at a level similar to that obtained with phenylalanine (not tabulated). Incorporation from the amino acid mixture was 70% maximal without added poly U, and was strongly inhibited by puromycin or Stage 21 fraction S.

Cell-free systems from frog larvae. Fractionation of homogenates of frog embryos and larvae from various developmental stages by the same procedures that yielded active amino acid-incorporating systems from adult frog liver resulted in preparations with low or negligible activity. Table III shows results with typical preparations from larvae of Stage 21, 1 day after hatching, from larvae of Stage 25 plus 6 days, about the time when a significant increase of total protein content is seen, and from larvae of Stage 25 plus 31 days, when total protein content has more than doubled. Incorporation levels are only slightly above the levels of 0 time controls. When larval M and pH 5 fractions were washed by reprecipitation to remove a possible soluble and reversible inhibitor, the washed fractions still showed only low ^{14}C incorporation capacities (not tabulated). Finally, combinations of frog liver fraction M or pH 5 fraction with the complementary washed or unwashed fraction from Stage 21 larvae gave incorporation levels no greater than those of the individual fractions separately (not tabulated).

RNA content of fractions. In view of the report that RNA components are readily bound and almost completely precipitated with the large particulate fraction in homogenates of frog eggs and embryos (7), the RNA content of fractions from homogenates of frog liver and of Stage 21 larvae was studied with an orcinol assay procedure (20). In 3 experiments, the mean total RNA content of adult frog liver was 8.37 mg/gm of wet weight; of this total, 1.92 mg was recovered in fraction M and 0.47 mg in the pH 5 fraction. For Stage 21 larvae, the mean total RNA content was 6.46 mg/gm of wet weight; fraction M contained 0.83 mg and the pH 5 fraction contained 0.18 mg.

Change of inhibitor concentration during development. The finding that fraction S from Stage 21 larvae inhibited incorporation by the frog liver system (Table IV) prompted a study of inhibitor concentration in preparations from various developmental stages. Preliminary experiments showed that inhibitory potency was concentrated in fraction S, and that the degree of inhibition was proportional to the amount of fraction S added. Table V summarizes the inhibitory potency of fraction S from embryos and larvae of various stages. Preparations from

TABLE V. Inhibitory Potency of Soluble Fraction from various Developmental Stages.[a]

Developmental stage	Ml of fraction S[b] added	Inhibition (%)
Stage 1	.01	90, 94[c]
Stage 21	.01	98, 99, 100[c]
	.001	73, 77, 78[c]
Stage 24	.01	95, 96[c]
Stage 25 + 6	.10	44, 50[c]
Stage 25 + 10	.10	54
Stage 25 + 25	.10	40
Stage 25 + 34	.20	69
Stage 25 + 46	.20	49

[a] The S fractions were obtained from homogenates of frog eggs and larvae of specified developmental stages as described under "Methods," and their inhibitory potencies determined by addition of designated amounts of S to the standard *in vitro* incubation system for L-phenylalanine-^{14}C incorporation into protein by frog liver fractions.

[b] One ml of S represents 0.25 gm of wet wt. of whole eggs or larvae.

[c] Values obtained with S fractions from different batches of eggs or larvae.

Stage 21 embryos were particularly potent; 0.001 ml of S, which caused about 75% inhibition, was derived from 0.25 mg of wet weight of embryo and contained about 3 μg of protein. Preparations from earlier stages, including unfertilized eggs (Stage 1), and from Stage 24 larvae were also very potent, but inhibitory potency was markedly reduced by Stage 25 + 6 days, and continued to decline slowly with age.

Characteristics of the inhibitor. For attempts to characterize the inhibitor and its mode of action, fraction S from Stage 21 larvae was used as the source of inhibitor; the standard frog liver fractions and phenylalanine incorporation system were used to assay inhibitor activity. The inhibitor activity was destroyed by heating in solution at 95°C for 20 min but was stable at −15°C for 3 months or at 2°C for 1 week. The inhibitor was nondialyzable and was not precipitated at pH 5.0 or pH 7.8 by ammonium sulfate at concentrations up to 75% saturation. The distribution of inhibitor activity in eluate fractions from calibrated Sephadex gel columns of various molecular exclusion limits indicated a molecular weight of between 10,000 and 50,000 for the inhibitor.

The inhibitor appeared to prevent phenylalanine incorporation rather than to cause breakdown of the final product of incorporation. This was indicated by the finding that if the inhibitor was added after 30 min of incubation, when incorporation was about 90% complete, the incorporation found 30 min later was about 90% of that found in the standard 60-min incubation without added inhibitor. A possible effect of the inhibitor on an essential cofactor was tested by incubating the cofactors, individually and in combination, with 0.02 ml of fraction S but without tissue fractions at 30°C for 30 min; the system was then heated at 95°C for 20 min to inactivate the inhibitor, and finally the other cofactors, phenylalanine-^{14}C and tissue fractions were added to begin the standard incubation procedure. The resulting incorporation values indicated that preincubation with the inhibitor did not markedly affect any essential cofactors, except for a partial (40–80%) inactivation of poly U. When assayed by McDonald's procedure (21), fraction S showed significant depolymerase activity with yeast soluble RNA or poly U as substrate.

Amino acid activation. A hydroxamate formation procedure (22) was used, with minor modifications, to assay the phenylalanine-activating capacity of fraction M plus pH 5 fraction. Each assay contained, in a total volume of 1 ml, the amount of fraction M plus pH 5 fraction obtained from 0.16 gm of wet weight of liver or Stage 21 larvae, and the amount of hydroxamate formed in 30 min was determined. In assays of 5 liver preparations, the mean value and standard deviation was 0.28 $\pm$ 0.03 μmoles hydroxamate formed, and for 4 preparations from Stage 21 larvae the value was 0.16 $\pm$ 0.01 μmoles. It therefore appeared unlikely that a deficiency of phenylalanine-activating capacity was responsible for the low level of phenylalanine-incorporating activity in larval preparations, relative to that in liver preparations. The phenylalanine-activating activity of frog liv-

er M plus pH 5 fraction was significantly inhibited when Stage 21 fraction S was added to the incubation system: 0.001 ml of S produced 17% inhibition (range 12–20%), while 0.004 ml produced 58% inhibition (range 48–68%).

Discussion. The time-course for increase of total protein content in developing frog embryos and larvae, which was determined as a base line for the present study, is consistent with the pattern previously reported (1). The present findings on the rate of *in vivo* uptake of amino acids from external medium and their incorporation into protein at various developmental stages are temporally consistent with the pattern of increasing protein content; a marked increase in rate of uptake and incorporation occurs at about Stage 24, shortly after active feeding begins and some days before a significant increase in total protein content was observed. From the data of Table II, it appears that restricted uptake of amino acid from the medium is a major factor in the low rates of *in vivo* incorporation seen for prefeeding larvae, as was previously (11,12) shown for prehatching frog embryos. The present results on *in vivo* incorporation of exogenous amino acids into protein therefore do not provide a measure of the capacity of prefeeding larvae to synthesize protein from endogenous amino acids.

The *in vitro* system from frog liver for incorporation of either phenylalanine or an amino acid mixture into polypeptide is similar in general characteristics to *in vitro* protein-synthesizing systems obtained from other species. It resembles the amino acid-incorporating systems obtained from *Xenopus* liver and ovary by Ford (23), but differences in preparative and assay procedures do not allow detailed comparison.

Any of several factors might be responsible for the low activities of preparations from frog larvae in the *in vitro* amino acid-incorporation assay. The embryos and larvae may possess inherently low protein-synthesizing capacities, compared to liver, but an appreciable activity would be expected, at least in older larvae which are feeding actively and show a rapid increase of total protein content. A loss or destruction of essential components during the preparative procedures may be a factor, but the content and recovery of protein, RNA and amino acid-activating capacity in homogenate fractions are not grossly different for liver and larvae. It is also possible that the *in vitro* assay conditions defined for the liver preparations are not optimal for larval preparations. Finally, regardless of other factors, the inhibitory component found in fraction S of embryo and larval homogenates could cause the low activities observed with cell-free preparations from larvae.

The nature and mode of action of the inhibitor remain obscure. However, the heat lability and high molecular weight of the material, coupled with the high potency of even a crude soluble fraction, suggest that the inhibitor is a catalytically active macromolecule, possibly an enzyme. It may be significant that the inhibitor concentration decreases sharply at about the time when the reserve materials of yolk become depleted. The site of inhibitor action appears to be some component of the protein-synthesizing apparatus present in the microsomal fraction or pH 5 fraction since, except for a partial inactivation of poly U, the inhibitor did not markedly deplete cofactors preincubated with it. The effect on poly U may reflect a primary attack by the inhibitor on some essential nucleic acid component. A nonspecific binding or precipitation of nucleic acid components is an unlikely mechanism of inhibitor action, because the amounts of nucleic acid components in the *in vitro* incubation system are vastly greater than the amount of inhibitor fraction that produces essentially complete inhibition. A specific binding with some RNA component is not excluded, but there is no evidence of such binding. Primary inhibition at the level of amino acid activation also cannot be excluded, although the degree of inhibition in this process was significantly less than the inhibition of the overall incorporation process.

Any possible physiological significance of the inhibitor remains uncertain. It appears

inconsistent that significant inhibitor activity is found even in preparations from late larval stages in which there is active *in vivo* incorporation of amino acids and a net accumulation of protein; however, the inhibitor concentration is markedly lower at the developmental stage when a significant increase in total protein content is first observed than at earlier developmental stages (Tables I and V). It is possible that the conditions of the *in vitro* incubation system permit expression of an inhibitory effect that is at least partially prevented under *in vivo* conditions. Alternately, the inhibitor may be present and functional *in vivo*, but only in certain cells or tissues, and at only certain developmental stages. Finally, the inhibitor activity may be an artifact of the preparative procedures, perhaps resulting from release or activation of a lytic enzyme. Even if the observed inhibitory activity is not involved in physiological control of protein synthesis, it is of interest that a powerful potential inhibitor is present in such high concentration at certain stages of embryonic development in the frog.

Summary. The capacity to carry out activities related to protein synthesis has been studied in developing frog embryos and post hatching larvae. Rapid incorporation of labeled amino acids from the external medium into protein could be demonstrated with intact larvae about 5 days before a net increase of total protein was found. Only minor incorporation of labeled amino acid into protein was obtained with cell-free preparations from frog embryos and larvae, even at developmental stages when the total protein content was increasing; however, these preparations did possess a high capacity for amino acid activation. In contrast, analogous cell-free fractions from adult frog liver contained an active system for incorporation of phenylalanine or of an amino acid mixture into protein; the characteristics of the frog liver system were similar to those of cell-free systems for amino acid incorporation described in other species. The low incorporating activity of cell-free preparations from frog embryos or larvae was related to the presence of a soluble, heat-labile component of high molecular weight that strongly inhibited amino acid incorporation by the cell-free system from frog liver. The concentration of this inhibitor in preparations from developing larvae decreased markedly at a developmental stage shortly before the larvae first showed a significant increase of total protein content.

The author is indebted to Dr. D. D. Brown and Dr. F. J. Finamore for helpful discussions. The technical assistance of Mrs. B. Carter and Mr. R. Taylor is gratefully acknowledged.

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Received Feb. 5, 1968. P.S.E.B.M., 1968, Vol. 128.

Renal Excretion of 3-O-Methyl-D-glucose* (33017)

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Recently the literature has revealed that many substances move bidirectionally across the renal tubules of mammalian kidney. These include most weak organic acids (1, 2), ions such as Na^+, K^+, Ca^{2+}, and PO_4^{3-} (3–6) and even D-glucose (7,8). Evidence has also been presented in our previous papers for the existence of a common renal carrier system for sugars which reabsorbs D-glucose and also has the capacity of secreting L-hexoses and 3-*O*-methyl-D-glucose (3MG). The movement of sugars in either direction was shown to be sensitive to the inhibitory action of phlorizin (9,10). The 3MG, the methyl analog of D-glucose, has been a very useful tool for studying sugar transport in several biological systems. This is principally due to the fact that mammalian organisms are incapable of metabolizing this sugar analog as they would D-glucose (11–13). The nature and direction of transport of 3MG across the intestinal mucosa (14,15), erythrocyte membranes (16) and Ehrlich ascites tumor cells (17) has been found to be very similar to that of D-glucose transport in each of these systems. However, in the sugar transport system of the renal tubules, 3MG behaves more similarly to L-glucose than D-glucose. This was interpreted by us as being due to similarities in the structural configurations of 3MG and L-glucose (9). Dissimilarity of 3MG transport and D-glucose transport has also been reported by Kleinzeller and his co-workers (18). They found that rabbit kidney slices did not accumulate 3MG, but did accumulate several other sugars including D-glucose.

Recently Stolte *et al.*, using the microperfusion technique of perfusing single renal tubules, found that 3MG was absorbed from the perfusate at approximately 50% of the rate determined for D-glucose absorption in rats (19). This suggests that species difference may also dictate the characteristics of 3MG transport in the kidney. It was the purpose of this investigation to reexamine the tubular transport of 3MG in both dogs and rats and to demonstrate correlations which might exist between the transport of D-glucose and 3MG, in order to add further evidence for our proposal of a bidirectional sugar carrier in the renal tubular epithelium.

Experimental Method. Material. Nonlabeled 3MG was purchased from Calbiochem (Calif.) and ^{14}C labeled 3MG from New England Nuclear Corp. (Boston). Thin-layer chromatography indicated that both substances used in these experiments were probably homogenous compounds since each gave single spots with identical R_f values. The recovery of radioactivity from 3MG-^{14}C spots was always in the range of 93–97%. Inulin was obtained from Nutritional Biochemicals Corp. (Cleveland) and was subjected to chromatography by the procedure of Merck (20). Single spots were obtained from inulin samples indicating the probable absence of other carbohydrates as contaminants.

Methods. Dogs anesthetized with pentobarbital and rats with "Inactin" (ethyl-1-methyl-propyl-malonyl-thiourea) were used in T value determination of 3MG. In all

* This work was supported in part by USPHS Grant AM-02217-08 and National Science Foundation Grant GB-4774.

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