accessible the substrate to the proteolytic component. It is not impossible that this preliminary reaction, taking place during the lag phase, could be the mucolytic process mentioned by BANGA and BALO[6]. We could verify that the adsorption mechanism holds for the dissolution of non-diazotised aorta-elastin too.

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Service of biological Chemistry, Faculty of Medicine, Paris, December 4, 1956.

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Résumé

Nous présentons une étude du mécanisme de l'élastolyse par une élastase partiellement purifiée du pancréas. La méthode utilisée consiste dans le dosage spectrophotométrique de l'«azoelastine» dissoute. Cette protéine n'est que très lentement hydrolysée par la trypsine et chymotrypsine cristallisées. L'élastase possède une activité protéasique considérable. L'enzyme est très rapidement adsorbée sur l'élastine. Après une incubation à pH 4,5 avec l'enzyme adsorbée, le temps de latence de l'élastolyse disparaît et la rapidité de la réaction devient proportionnelle à la concentration en élastase.

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STUDIORUM PROGRESSUS

The Metabolism of the Proteins of the Brain*

By A. LAJTHA, S. FURST**, and H. WAELSCH

Of the major components of the brain, least attention has been paid to the protein constituents. The large concentration of lipids and the process of myelination, unique for the nervous tissue and so easily visualized by histological techniques, have for many years attracted the interest of biochemists. With the development of our understanding of the role of carbohydrates in the energy metabolism of living tissue, the last 25 years witnessed an ever increasing concern with this aspect of intermediary metabolism of the brain. Only recently have the amino acids and proteins of the central nervous system been subjected to more intensive study, but these investigations have been and are essentially analytical in nature. Hardly any other organ system offers as many fascinating and far reaching implications for the function of its component proteins as does the nervous system.

This laboratory has for many years been interested in the metabolism of the amino acids and proteins of the nervous system, and we should like to summarize in this report our studies dealing with the turnover of the proteins of the whole brain, of different parts of the brain, and of different cell fractions[1]. Incidental to these investigations, studies of the penetration of lysine into the brain of young and adult animals as well as observations on protein metabolism in the immature brain have been made.

The study of the turnover of brain proteins by the determination of the rate of uptake of an isotopically labelled amino acid is made difficult by the existence of the blood-brain barrier which decreases the rate of transfer of many metabolites from the circulating blood to the brain. Some compounds are, for all practical purposes, excluded from the brain[2]. For example, no increase of the glutamic acid concentration in brain could be detected after a 30 fold increase of this amino acid in the blood. On the other hand, a significant, although small, increase of the glutamine concentration in brain was found after intravenous administration of the amide to rats or mice[3]. It is not surprising that isolated observations of lack of incorporation of amino acids, parenterally administered, led to the conclusion that the brain proteins have a very slow turnover[4]. When, on the other hand, amino acids were introduced directly into the subarachnoidal spaces, in order to avoid the blood-brain barrier, considerable incorporation into proteins was observed[5].

The design of the experiments.—For reasons to be discussed below, it appeared desirable to select the physiological path of supply of amino acids to the brain, i.e., from the circulating blood. Lysine was chosen as the test amino acid, there being reason to believe that this basic amino acid would penetrate the blood-brain barrier at a faster rate than an acidic or neutral one[6]. The technique followed, with minor variations, in the experiments to be reported, was as follows:

Lysine C^{14} was injected intravenously or intraperitoneally into mice and monkeys, and the brains, livers and other organs were removed after definite time intervals. The protein fractions obtained by precipitation with trichloroacetic acid and extraction with organic

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[2] H. WAELSCH in *Biochemistry of the Developing Nervous System* (H. WAELSCH ed., Academic Press, Inc., New York 1955).

[3] P. SCHWERIN, S. P. BESSMAN, and H. WAELSCH, J. biol. Chem. *184*, 37 (1950).

[4] H. BORSOOK and C. L. DEASY, Ann. Rev. Biochem. *20*, 209 (1951).

[5] F. FRIEDBERG, H. TARVER, and D. GREENBERG, J. biol. Chem. *173*, 355 (1948). – M. K. GAITONDE and D. RICHTER, Biochem. J. *59*, 690 (1955).

[6] H. WAELSCH in *Biochemistry of the Developing Nervous System* (H. WAELSCH ed., Academic Press, Inc., New York 1955). – P. SCHWERIN, S. P. BESSMAN, and H. WAELSCH, J. biol. Chem. *184*, 37 (1950).

solvents were measured for their radioactivity, and, after hydrolysis, the lysine content was determined by microbiological assay for calculation of specific activities. Similarly, in the nonprotein fraction of each organ, radioactivity and lysine content were determined. In control experiments it was ascertained that during the experimental period less than 1% of the radioactivity administered as lysine was found in amino acids other than lysine. Postmortem changes owing to manipulation of the excised organs did not influence the results.

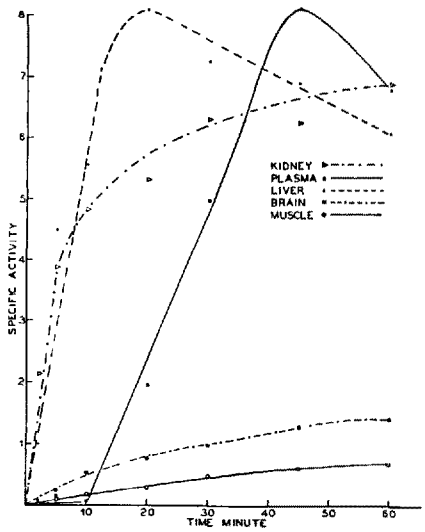

Fig. 1. – Specific activity of protein bound lysine of various organs (counts/ γ lysine/min) after intravenous injection of lysine C^{14} in adult mice.

The results of a typical experiment in which groups of mice were sacrificed at different time intervals after intravenous injection of radioactive lysine are shown in Figure 1. It will be noted that the experimental periods chosen are in minutes. This is in contrast to experiments employing considerably longer time intervals in which the half life time of proteins was calculated from the decay of radioactivity or N^{15} content of a protein after administration of a labelled amino acid[4]. These latter experiments supply data for calculation of the turnover of the bulk of the slowly metabolized proteins of an organ. They do not, however, offer information on rapidly metabolized proteins from which the labelled amino acid has disappeared in the experimental period and has been diluted out by the pool of non-labelled free amino acids. On the other hand, experiments of the type described in the present communication must be cautiously interpreted because of the rapid changes occurring during the short-time interval. The results of the experiment shown in Figure 1 demonstrate that within minutes after administration, labelled lysine appears in the brain proteins despite the fact that the amino acid had been administered without circumvention of the blood-brain barrier.

The renewal of the amino acid pool as measured with isotopic lysine. – According to the prevailing views proteins draw their constituent amino acids from the free amino acids of the nonprotein fraction. The calculation of turnover rates of the proteins of an organ requires knowledge of the specific activity of the test amino acid in the amino acid pool of the organ[7]. In our experiments,

[7] O. B. Henriques, S. B. Henriques, and A. Neuberger, Biochem. J. *60*, 409 (1955).

Table I. – Flux (F) of free lysine into mouse brain and liver
γ lysine/g fresh tissue/min

Duration of experiment minutes	Brain				Liver		
	Exp. III Adult	Exp. V Adult	Exp. VII Young	Exp. VIII Young	Exp. III Adult	Exp. VII Young	Exp. VIII Young
2	2·3				29		
3		0·7					
5		1·3	1·2	1·1	10		
10			1·5	1·1	20	24	63
20	5·2				6·9	8·5	
30	1·5				6·3		
45						11	8·4

Adult mice: 120 days old; young mice: 10 days old.
$F = C\,(dSb^{***}/dt)/Sa - Sb$ where $C = \gamma$ free lysine in g fresh tissue; Sa = specific activity of plasma free lysine; Sb = specific activity of organ free lysine.
*** Estimated graphically

considerable radioactivity was found in the free amino acid fraction of the brain of mice even when the animals were killed 2 min after the administration of the isotopic amino acid (Fig. 2). This observation suggests that the blood-brain barrier is permeable to lysine. The transfer coefficient or flux of free lysine from blood to the brain of mice may be estimated on the basis of the concentrations of isotopic lysine found in the plasma and in the nonprotein fraction of the organ (Table I). These estimates can only be approximate since they are based on the rapidly changing isotope concentration in the plasma during the first minutes of the experiment. The values are expressed in γ of lysine transferred per minute from blood to brain and show a considerable range varying from 0·7 γ to 5·2 γ/g tissue/min. From these values the time interval in which one half of the free lysine was replaced was calculated. It was found not to exceed 45 min for both young and adult mice. The same calculation when applied to replacement of half the free lysine of liver leads to values not greater than 10 min.

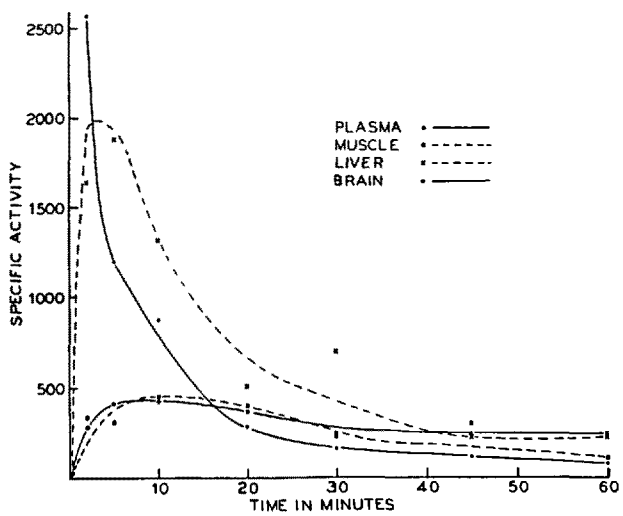

Fig. 2. – Specific activity of free lysine of various organs (counts/γ lysine/min) after intravenous injection of lysine C^{14} in adult mice.

In addition to exchange, or turnover rate, the problem of net uptake presents itself. Accordingly, it was attempted to increase the concentration of the free lysine

Table II.—Half-life time (days) of proteins of mouse organs

Time	Brain		Liver		Muscle	
After injection in minutes	Adult	Young	Adult	Young	Adult	Young
2	2·8		0·90		3·5	
5	3·5	3·4	1·25	1·73	7·6	2·4
10	5·5	2·2	2·57	1·10	7·6	1·7
20	6·2	3·0		2·68	10·4	2·5
30	6·9				13·2	
45	10·4	2·4			16·6	2·3
60	15·2				23·6	

Half-life time was calculated according to the following equation[8]:

$$\tfrac{1}{2} \text{ life in days} = C \times \ln 2/F \times 1440$$

$C = \gamma$ lysine/mg dry protein; F = flux of lysine (γ lysine incorporated/mg protein/min).

of the brains of young (10 days old) and adult mice by intravenous injection of large amounts of nonisotopic amino acid. By raising the concentration of the free lysine of the plasma 25–50 fold, increased brain concentrations were effected. These averaged 100% in the young animal, while the significance of the changes in lysine concentration of the brain of adult animals is questionable.

Thus while the experimental evidence is not conclusive, it suggests that in brains of both young and adult mice the free lysine is *renewed* at comparable rates although larger *net uptake* of lysine can be induced in the brain of the former.

The half-life time of the brain proteins.—The flux of lysine from the nonprotein nitrogen fraction into the proteins was calculated on the basis of the specific activity of the free lysine and the protein-bound lysine of the brain and other organs at given times. These data are expressed as half-life time in order to facilitate comparison with values recorded in the literature (Table II). In the experiments with adult mice the calculated half-life time of the protein increased with each increasing time interval between administration of the amino acid and the sacrifice of the animal. The same relationship was found for the half-life time of muscle and liver protein. It should be pointed out that all derivations developed for calculation of half-life of turnover time of proteins rest on the assumption of a homogeneous system in which the component proteins have similar turnover rates. Such a situation may be simulated when the turnover of protein is determined from decay curves since here the bulk of the proteins with a slow turnover rate are measured, and these may appear as a homogeneous system. In experiments of short duration, the ascending portion of the activity time-curve is measured and in these, the various rapidly metabolized protein fractions will be prominent. The spectrum of turnover values obtained in our experiments reflects the metabolic inhomogeneity of the protein of the organs investigated, a fact which by itself hardly needs experimental verification.

When the same experiments were carried out with immature mice it was found that the fractions of slowly metabolized proteins were missing, while the turnover rates of the rapidly metabolized proteins were little altered (Table II).

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Table III.—Half-life times of proteins in monkey brain areas

Areas of brain	Half-life time in days calculated from different time points		
	5 min	10 min	45 min
Cord	10·6	9·5	18·5
White	3·9	4·0	8·7
Medulla-Pons	6·6	6·7	13·7
Thalamus-Hypothalamus	6·1	7·8	16·8
Cerebellum	5·4	7·6	14·1
Cerebral cortex	5·5	6·7	12·7

Further insight into the distribution of proteins with varying turnover rates may be obtained by the study of the metabolic activity of the proteins of the separate intracellular fractions, as well as of the metabolic picture characteristic of different parts of the organ. In an organ which is relatively homogeneous morphologically, such as the liver, the proteins of different turnover rates will be distributed throughout the organ. In an organ as morphologically inhomogeneous as the brain, the concentrations of proteins of different turnover rates may vary considerably from one area to another.

The turnover of proteins in different parts of the brain. In studying the turnover rate of proteins of different parts of the brain, adult Macaque monkeys (*Macacca Irus*) were used (Table III), since in these animals the ratio of brain to body weight is particularly high. Although care was taken in the preparation of the designated areas of the brain, inclusion of a certain amount of tissue from neighbouring areas could not be completely avoided. The highest rate of incorporation was found in the corpus callosum followed by the cortex. The spinal cord showed the lowest rate of incorporation. It is unquestionably striking and unexpected to find the highest rate of incorporation in the white matter of the corpus callosum and its radiation where fibres and oligodendroglia cells predominate. Without further study this finding should not be taken to mean that the turnover of the neuronal protein of the cortex is slower than that of the glial elements since no estimate of the contribution of neuronal or glial proteins to the over all picture can be made at the present time. Also it has been suggested that the site of synthesis of at least some of the axonal proteins is in the neuronal cell[9] and it may therefore not be justified to relate the rate of incorporation of amino acids into the proteins of the white matter to the amino acid pool of this area. Regardless of the ultimate answer to questions of the distribution of proteins of different turnover rates between neuronal and non-neuronal elements the various areas of the brain contribute to the picture of inhomogeneity of turnover rates found in the analysis of the whole brain.

Turnover of the proteins of different cell fractions.—The cerebral cortex of Macaques, at various time intervals after the injection of isotopic lysine into the animals, was homogenized in isotonic sucrose solution and the homogenate fractionated by centrifugation[10]. The relative homogeneity of the fractions was confirmed by electron microscopy. Nevertheless, no definite statement as to the absolute purity of the individual fractions is attempted at this time. Of particular interest are those sediments which are obtained at high centrifugal forces—the microsomal fractions of cells. After removal of fractions at 800, 1500, and 12,000 × g, the remaining supernatant

[9] P. WEISS and H. B. HISCOE, J. exp. Zool. *107*, 315 (1948).
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Table IV
Incorporation of lysine C^{14} into different cell fractions of brain cortex

Centrifugation		Counts/mg lysine/min.			Composition of Fraction
		Duration of Experiment minutes			
No. of gravities	Time in min.	5	15	30	
800	10	86	190	500	Cell debris, whole cells, nuclei
1500	10	140			Nuclei, large mitochondria
12000	15	270	200	394	Mitochondria
23000	30	390	670	790	Large microsomes
105000	30	540	860	1388	Small microsomes
105000	120		310	1346	Small microsomes
S		170		570	Supernatant fluid

fluid was subjected to succeeding centrifugations at 23,000 and 105,000 × g and the pellets collected. The specific activity of the lysine of the protein of these particulate fractions, as well as that of the protein remaining in the supernatant fluid, was determined (Table IV). In agreement with the results obtained with liver microsomes by other investigators[11], the microsomal fractions of brain incorporated lysine at a higher rate than other cell fractions. It should also be noted that the particles of the microsomal fractions obtained by centrifugation at higher speeds and for an extended period of time showed a higher specific activity than the microsomal fraction collected first. This finding agrees well with the observation that the smaller particles of the microsomal fraction of liver have a higher rate of incorporation than the larger microsomes in *in vitro* experiments[12]. The proteins of the different cell fractions, therefore, add to the observed inhomogeneity of turnover rates of the brain proteins.

Discussion.—In approaching the problem of amino acid and protein metabolism of the central nervous system, the complexity of the organ presents considerable difficulties for the design of pertinent experiments. It should be recalled that the problem of the blood-brain barrier is only partially understood. While it is operative in the largest portion of the nature brain, there are areas in which its activity appears considerably diminished, such as the choroid plexus, the neural lobe and infundibulum of the pituitary, the pineal body, the area postrema, etc.[13]. Quantitatively, the absence of a blood-brain barrier in these structures may not seriously influence the uptake of substances when larger areas of the brain are studied. However, the cerebrospinal fluid originating in the choroid plexus undoubtedly must be considered because of the contribution it makes to the composition of the interstitial fluid through which any substance taken up by the cells has to pass. Substances introduced directly into the subarachnoid spaces circumvent the blood-brain barrier and follow the path taken by the cerebrospinal fluid. From the ventricles at least some will penetrate the adjacent parts of the brain and it cannot be assumed that their subsequent distribution in the brain will be the same as when offered from the circulating blood. This is supported by the observation that the replacement of chloride in the cortex 3 h after the injection of bromide, iodide, or thiocyanate into the cisterna magna was found to be only one-fifth of that replaced in the cisternal fluid, while after intravenous injection, chloride replacement was the same in both cortex and cerebrospinal fluid[14]. Experiments in which radio phosphorus was introduced into the cerebrospinal fluid showed that the radioactivity was not distributed uniformly throughout the brain, but was greatest in the areas adjacent to the ventricles and decreased progressively in the more distant parts[15].

Although lysine has a high exchange rate which permits the renewal of the free lysine of the brain within 1 h, the fully developed blood-brain barrier interferes with any extensive net uptake of the amino acid by the brain. This finding demonstrates that the blood-brain barrier also regulates the net uptake of small molecular basic substances, and again emphasizes its role in maintaining brain homeostasis. On the other hand, the immature brain permits a considerably greater net uptake of lysine from the circulating blood than does the mature brain, despite the fact that the exchange rates for the two are similar. This raises the question whether or not the same mechanisms govern both net uptake, and exchange of the amino acid. The fact that no net uptake of glutamic acid by the mature brain from the circulating blood could be demonstrated[3] prompts the question as to whether the rate of exchange for acidic amino acids will be as high as for lysine. Differences in the exchanges rates of different amino acids would mean that the free amino acid pool of the brain is renewed at a specific rate for each amino acid or group of amino acids.

While half of the free lysine of the mouse brain is replaced in less than 45 min, the replacement of half of the potassium content of the rat brain may be calculated to require approximately 24 h[16]. It may be assumed that a similar relationship also holds true in the same animal. In view of the report that lysine can replace potassium in animals raised on a potassium-free diet[17], the interesting possibility arises that the movements of the basic amino acids and of potassium may be interdependent.

In computing the turnover rates of the proteins of an organ, the specific activity of the labelled free amino acid in the organ must be taken into account. In addition to its high exchange rate between circulating blood and brain, lysine has the advantage over amino acids such as glycine[7] or methionine[18] in that it is converted only slowly to other amino acids, and therefore calculations of turnover rates based on its incorporation into proteins are simplified. Very recently experiments were reported in which methionine S^{32} was used for the study of protein turnover[19]. In these experiments labelled methionine was introduced into the cisterna magna in a concentration 100 fold that of the free methionine of the brain. By assuming the resulting activity of the methionine pool to be close to that of the administered methionine, the authors felt justified in omitting the determination

[11] E. B. Keller, P. C. Zamecnik, and R. B. Loftfield, J. Histochem. Cytochem. *2*, 378 (1954).

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of the specific activity of methionine in the nonprotein nitrogen fraction. It is of interest to compare their turnover rates with those found in our experiments. While in our studies, progressively increasing time intervals between injection and killing of the animal resulted in progressively decreasing turnover rates, GAITONDE *et al.*[19] found no similar relationship and calculated a mean half-life for the proteins of 13·7 days ± 4·1 (s.d.). Although these authors carried out short interval experiments comparable in duration to ours, the relative constancy of the turnover rate found by the use of radioactive methionine makes one suspect that, with intracisternal administration, mainly the turnover of slowly metabolized brain proteins neighbouring the ventricular cavities was measured (see above).

The finding of the different turnover rates for the proteins of brain, liver and muscle depending on the time interval between administration of lysine and sacrifice of the animal gives an indication of the metabolic inhomogeneity of the component proteins. The turnover rate of liver proteins also decreases with increasing experimental period despite the fact that this organ is relatively homogeneous containing about 60% parenchymatous cells. It is, therefore, not surprising that the same phenomenon is observed in an organ such as the brain which is characterized by a heterogeneous cell population and in which the ratios of neuronal to nonneuronal elements vary markedly from one area to another. The complexity is considerable even within a 'homogeneous' part of the brain such as the cortex, in which the proteins of the perikarya, of the dendrites, of the glia, of the axons, and probably also some mucoproteins of the 'groundsubstance', are all present. Each part of the brain contains proteins with high and low turnover rates; their quantitative distribution is probably different from part to part, and from structure to structure.

The growth increment of mouse brain between the 10th day of life and maturity is small. Nevertheless, an indication of the mechanism of growth in brain and other organs as well is given by the observation that in young animals the absence of the slowly metabolized proteins is not associated with an increase in the turnover rate of the fast' fraction. The turnover rate of the 'fastest' protein fraction is apparently rapid enough so as not to be changed significantly by the additional contribution due to the net synthesis of protein in the growing organ. A similar observation was made in an investigation of rate of fatty acid synthesis during growth, when no increase in the rate of deuterium incorporation was noted during the period of myelination[20].

The incorporation of lysine into the proteins of an organ can be due to intracellular protein metabolism or to synthetic processes associated with the replacement of dead cells. The latter mechanism would imitate a dynamic state in those organs in which the continuous death and replacement of a considerable number of cells is the rule, a situation which does not hold for the neuronal elements of the brain. It is very unlikely that the replacement of glial elements is fast enough to account for the high turnover rate of brain proteins. Thus, the rapid incorporation of lysine and methionine argues strongly for a dynamic state of brain proteins. Of particular interest in studies of protein synthesis and degradation are those fractions which have a particularly fast turnover. The shortest half-life time calculated for proteins of the whole brain was 2·8 days. This is somewhat slower than the fastest fraction of the liver, which, in the same experiments, had a half-life time of 1·0 days (Table II). These figures give only an indication of the rapidity of the turnover of some protein fractions of the two organs. If we take the values found for the microsomal proteins (which are probably also a mixture of proteins of different turnover rates), we find that the rate of lysine incorporation into this fraction is about 5 times higher than it is into the proteins of the whole homogenate (Table IV). It appears, therefore, that certain protein fractions of the brain are renewed in a matter of hours.

Histochemical and biochemical evidence for the participation of proteins in the functional activity of brain is scarce[21]. The findings reported in this summary raise the significant question as to whether the spectrum of rates of protein metabolism is the secondary expression of the general energy and nutrient metabolism of this organ, or whether its variety and range contribute to a physiological basis for the functional capacity of the central nervous system.

Zusammenfassung

Mit Hilfe von isotopem Lysin wurde der Austausch von freiem Lysin zwischen Blut und Gehirn, Leber und Muskel und die Umsatzrate der Eiweisskörper dieser Organe bestimmt.

Im Zusammenhang mit den erhobenen Befunden wird die Funktion der Blut-Hirnschranke und die Erneuerung der freien Aminosäuren und der Proteine im Gehirn und in anderen Organen erörtert.

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COGITATIONES

The Biosynthesis of Penicillin

By K. GANAPATHI*

A theory of biosynthesis of penicillin should define: (i) the immediate precursors partaking in the biosynthesis: (ii) the way the precursor units react mediated by enzyme systems to yield penicillin; (iii) the pathways of synthesis of these precursors themselves from the media constituents; and (iv) the specific differences in biochemical terms between the poor penicillin-producing strains of *Penicillium chrysogenum* and the high yielding ones. The knowledge available at present is far from complete. An attempt is made here to marshal the data available and evolve a coherent picture which can form the basis for further work. The significant facts that make the studies difficult are: (1) the biosynthesis of penicillin by *P. chrysogenum* appears to be of no special significance in the economy of the mould itself; (2) in the penicillin-producing phase, the mould synthesises only about 0·01 to 0·03% of its weight of penicillin per hour; (3) even under the best of conditions, the quantity of penicillin produced is only 1 to 5% of the major media chemical used; and (4) the precursors that take part in penicillin biosynthesis are used extensively for other synthetic activities, such as the formation of cellular proteins.

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