observed as fine granular precipitates associated with ribosomes lining on the outer surface of endoplasmic reticulum in the vicinity of the nucleus. In the cisternae of the endoplasmic reticulum, nucleoplasm, mitochondria and free ribosomes, no reaction products are observed. As shown in Figure 2, by 9 h after infection the stained ribosomes increase in number. As seen in an inset of Figure 2, the surface of ribosomes thus stained is actually covered with reaction products. Beside those seen in the membrane-bound ribosomes, tiny masses of reaction products are dispersed among the cell organelles in the cytoplasmic matrix at this stage. No reaction products are associated with cytoplasmic nucleocapsid filaments. Figure 3 shows part of an infected HeLa cell surface at 13 h after infection. The reaction products are mostly restricted in the spikes on the outer surface of budding particles. By accumulation of the reaction products, the layer of spikes increases in width to ca. 300–400 Å. Both the unit membranes beaneth the spikes and the tubules of nucleocapsid are not stained. In control experiments, no reaction products were observed anywhere in the cells. The specificity of the antigen-antibody reaction was further supported by the absence of the reaction products in the tubular nucleocapsid both in the cytoplasm and the budding particles.

Discussion. The NDV-V antigen consists of two major components, hemagglutinin and neuraminidase[11]. Recently, IINUMA et al. found biochemically that the synthesis of both the components was initiated by 4 h and reached maximum by 9 h after infection in HeLa cells[12]. Judging from their results, the present findings may be interpreted as indicating that the initial sites of synthesis of the components are in the membrane-bound ribosomes. Furthermore, the antigen thus synthesized may be released from the bound ribosomes into the cytoplasmic matrix, not into the cisternae of the endoplasmic reticulum, in contraction to the present concept of secretory protein synthesis[13]. Why the initial synthesis does not involve the free polysomes is a further problem.

Zusammenfassung. Nach indirekter, peroxidasekonjugierter Antikörpermethode wurden elektronenmikroskopisch mit Newcastle-Disease-Virus infizierte HeLa-Zellen geprüft, und in den frühen Infektionsstadien an den Ribosomen des endoplasmatischen Retikulums solcher Zellen und in den späten Infektionsstadien an den «Spikes» des spriessenden Viruskörperchens das Antigen der Virusoberflächen gezeigt.

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STUDIORUM PROGRESSUS

The Measurement of Transketolase Activity in Heart Preparations

Our studies, directed at quantitating the role of the pentose phosphate pathway of glucose metabolism during the interval of developing myocardial hypertrophy in the rat and the rabbit, have necessitated the examination of changes in the activity of each of the enzymes of the pentose phophate pathway. In particular our attention has been directed to the role of transketolase (EC 2.2.1.1) where according to the experimental results of MEERSON et al.[1], a significant increase (60%) in the activity of this enzyme occurred in rabbit heart following the development of myocardial hypertrophy, experimentally induced by stenosis of the aorta. It was implied[1] that the increase in transketolase activity reflected the activation of the pentose phosphate pathway reactions to provide ribose 5-phosphate for the increased nucleic acid metabolism associated with the enhanced protein synthesis in hypertrophy. The method used[1] for the measurement of transketolase activity was that of BRUNS et al.[2] where sedoheptulose 7-phosphate (see Equation 1) was determined colourimetrically after a 1 h incubation of ribose 5-phosphate with the tissue extract.

Equation 1: xylulose 5–P + ribose 5–P $\rightleftharpoons$ sedoheptulose 7–P + glyceraldehyde 3–P

We have found that the colourimetric method for the measurement of transketolase[2] in heart extracts was unsatisfactory for this purpose. The principal objections to the application of the method[2] are a) it is not specific for sedoheptulose 7-phosphate while ribose 5-phosphate and other pentose phosphates contribute to the colour yield and thus may act to overestimate sedoheptulose 7-phosphate and therefore the activity of transketolase. b) It has to be assumed that in the tissue preparation being assayed the enzymes ribulose 5-phosphate 3-epimerase (EC 5.1.3.1) and ribose 5-phosphate isomerase (EC 5.3.1.6) are present and of sufficient activity so that they are not rate limiting for the reaction of Equation 1 to proceed. c) Finally it is essential that transaldolase (EC 2.2.1.2) or other enzymes which will react with sedoheptulose 7-phosphate are absent from the reaction mixture, since the presence of these enzymes would lead to low estimates of transketolase activity.

RACKER[3] has outlined 3 assays for transketolase which may be used with highly purified enzyme preparations,

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but has emphasized that these assays are not equally suitable for transketolase determinations in crude preparations. The first of these assays depends upon the formation of glyceraldehyde 3-phosphate from xylulose 5-phosphate in the presence of an acceptor aldehyde (e.g. equation 1). The formation of glyceraldehyde 3-phosphate is measured either with triose phosphate isomerase and L-α-glycerol phosphate dehydrogenase by following the oxidation of NADH or by the reduction of NAD in the presence of glyceraldehyde 3-phosphate dehydrogenase. Because the product of the reaction is removed as soon as it is formed and thus escapes side reactions RACKER[3] concluded that this assay was particularly suitable for transketolase measurements in crude preparations. The second assay is similar to that described by BRUNS et al.[2] and depends on the formation of sedoheptulose 7-phosphate which can be measured colourimetrically. Although it has been frequently used with crude enzyme preparations, it was not recommended[3] as a quantitative measure of transketolase, e.g. in the presence of transaldolase which removes the products of the transketolase reaction. The third assay depends on measurements of the disappearance of either donor substrate or acceptor aldehyde. Variants of this procedure include measurements of the disappearance of fructose 6-phosphate or of other ketol donors in the presence of excess acceptor aldehyde, or of the disappearance of glyceraldehyde 3-phosphate in the presence of excess donor substrate. RACKER[3] concluded that the third method of assay was usually unsuitable for crude enzyme preparations because of numerous side reactions which occurred with the substrates.

Thus of the methods described by RACKER[3] for transketolase assay only one method would appear to be satisfactory for crude tissue preparations and while this method is satisfactory for most tissues it has not been applied to heart (for example, reference[4]).

In this paper a comparison of methods is made for the determination of transketolase activity in heart preparations. A contrast of the levels of transketolase activity in heart when measured by the colourimetric method of BRUNS et al.[2] and specific enzyme assay procedures (Table) showed that the colourimetric procedure gave irregular results which were always an overestimate of transketolase activity. A new method is proposed involving the substrates hydroxy-pyruvic acid and erythrose 4-phosphate and the rate of transketolase activity is measured from the rate of formation of fructose 6-phosphate.

For the experiments described in this paper crystalline yeast transketolase was used as reference and was obtained from Sigma Chemical Co. (type IV, 20 units[5]/mg, and 0.45 mg/ml). A sample of 0.001 ml was used for the measurement of transketolase activity. Myocardial preparations were made from rat and rabbit hearts which were homogenized in 10 volume of icecold 0.25 *M* glycylglycine-KOH buffer pH 7.4. The homogenate preparation was centrifuged at 100,000 *g* for 30 min and the transketolase activity was measured on the supernatant fluid using method (V). A partially purified preparation of rat heart transketolase was made as described by NOVELLO and MCLEAN[4] for the preparation of rat liver transketolase to give a preparation free of NADH oxidase, ribulose 5-phosphate 3-epimerase, transaldolase and fructose 1,6-diphosphatase (EC 3.1.3.11).

Five methods were compared for the determination of transketolase activity. Method (I) involved the reaction of Equation (1) and the sedoheptulose 7-phosphate was determined colourimetrically[2]. Method (II) involved the same reaction (Equation 1) and glyceraldehyde 3-phosphate was determined enzymatically by the following procedure. Glycylglycine-KOH buffer, pH 7.4, 5 mmole was added to each of two cuvettes together with 0.5 μmole of $MgCl_2$, 0.05 μmole of thiamin pyrophosphate (Nutritional Biochemical Corp.), 0.5 μmole NADH, 0.05 ml of L-α-glycerophosphate dehydrogenase (EC 1.1.1.8) 5 mg/ml, 0.001 ml of triosephosphate isomerase (EC 5.3.1.1) 2 mg/ml, and 1 μmole of xylulose 5-phosphate (Sigma, grade III) in a total volume of 2.20 ml. The reaction was commenced by the addition of 1 μmole of ribose 5-phosphate (0.05 ml) to the test cuvette and the extinction change followed at 340 nm and 30°C for 20 min in a double beam recording spectrophotometer. Method (III) involved the reaction of Equation (2).

Equation 2: xylulose 5-P + erythrose 4-P $\rightleftharpoons$ fructose 6-P + glyceraldehyde 3-P and the glyceraldehyde 3-phosphate was determined enzymatically.

The reaction mixture was identical to (II) except that the reaction was commenced by the addition of 1 μmole of erythrose 4-phosphate (0.05 ml) to the test cuvette.

Method (IV) involved the reaction of Equation 2 and fructose 6-phosphate was determined enzymatically by the following procedure. Glycylglycine-KOH buffer, pH 7.4, 5 mmole was added to each of two cuvettes together with 0.5 μmole of $MgCl_2$, 0.05 μmole of thiamin pyrophosphate, 0.5 μmole of $NADP^+$, 0.005 ml of glucose 6-

[4] F. NOVELLO and P. MCLEAN, Biochem. J. *107*, 775 (1968).

[5] A unit of enzyme activity is defined as the amount of enzyme that converts a micromole of substrate to product in 1 min under optimal conditions of assay at 30°C.

Comparison of methods for the determination of transketolase activity

Method	Catalytic activity of transketolase				
	I	II	III	IV	V
Rat heart transketolase (units/mg protein) (partially purified)	0.51 ± 0.18 (4)	0.30 ± 0.02 (4)	0.20 ± 0.04 (4)	0.18 ± 0.02 (4)	0.026 ± 0.003 (4)
Yeast transketolase (units/mg protein)	19.5 ± 1.2 (3)	20.9 ± 0.2 (5)	14.3 ± 0.3 (5)	12.9 ± 0.3 (5)	2.6 ± 0.05 (4)
Rat heart extract (milli-units/g fresh wt.)	103 ± 26 (3)	—	—	51.0 ± 9.0 (9)	4.78 ± 0.07 (9)
Rat heart extract, 4 days after aortic constriction (milli-units/g fresh wt.)	—	—	—	24.0 ± 3.2 (6)	2.09 ± 0.11 (6)
Rabbit heart extract (milli-units/g fresh wt.)	68 ± 1.0[a]	—	—	35.8 ± 4.7 (3)	2.98 ± 0.09 (3)

The results shown are the means ± S.D. for the numbers of determinations shown in parentheses. [a] The results of MEERSON et al.[1].

phosphate dehydrogenase (EC 1.1.1.49) 1 mg/ml, 0.005 ml of glucose phosphate isomerase (EC 5.3.1.9) 2 mg/ml and 1 μmole of xylulose 5-phosphate in a total volume of 2.20 ml. The reaction was commenced by the addition of 1 μmole of erythrose 4-phosphate (0.05 ml) to the test cuvette.

Method (V) involved the reaction of Equation 3.

Equation 3: Hydroxypyruvate + erythrose 4-phosphate ⟶ CO_2 + fructose 6-phosphate

where fructose 6-phosphate was determined enzymatically. The reaction mixture was identical to (IV) except that 1 μmole of hydroxypyruvate (0.05 ml, lithium salt) was used in place of xylulose 5-phosphate.

Results and discussion. The data of the Table show the levels of transketolase activity measured by the 5 different methods. Methods (II) and (III) were found to be unsatisfactory for the measurement of the activity of the enzyme in tissue extracts of rat and rabbit heart because of the relatively high activity of NADH oxidase (rat, 0.25 ± 0.03 units/g fresh wt. and rabbit 1.65 ± 0.24 units/g fresh wt.). As the activity of transketolase in heart extracts was low (Table) the high activity of NADH oxidation effectively prevented the use of methods (II) and (III) which involved either the oxidation of, or the production of NADH from the coupled assay system for glyceraldehyde 3-phosphate[3]. It is often possible to remove or significantly lower NADH oxidase activity of tissue extracts by preparation of the extracts in 0.25 *M* sucrose or 0.15 *M* KCl and centrifuging the homogenate at 100,000 *g* for 1 h. These treatments failed to yield preparations in which NADH oxidase was affected. Anaerobic conditions did not appreciably prevent NADH oxidation (NADH oxidase activity under anaerobic conditions was found to be 0.1 ± 0.08 units/g fresh wt. for rat heart preparations).

The respiratory inhibitors, KCN and rotenone failed to inhibit the oxidation of NADH by the heart extracts. Potassium cyanide at concentrations from 5 to 15 m*M* was without effect on the rate of oxidation of NADH by the heart extracts, while concentrations of KCN greater than 5 m*M* markedly inhibited the coupled assay system used for the measurement of transketolase (cystalline yeast preparation) by methods (II) and (III) (Table). Concentrations of rotenone (0.4 to 1.0 m*M*) did not decrease the rate of NADH oxidation.

Comparison of the values for transketolase activity in partially purified rat heart enzyme preparation and heart extracts indicated that the application of Method (IV) (Table) caused an overestimate of the activity of the enzyme in tissue extracts. In method (IV) the fructose 6-phosphate in the reaction catalysed by transketolase (Equation 2) was determined using a coupled assay procedure to produce NADPH. The overestimate of the activity of the enzyme by this method resulted from the presence of triose phosphate isomerase, aldolase (EC 4.1.2.b) and fructose 1,6-diphosphatase in heart extracts, which act in combination to catalyse the formation of fructose 6-phosphate from glyceraldehyde 3-phosphate, the co-product of the reaction used in method (IV). The activity of fructose 1,6-diphosphatase in the rat heart extracts was found to be approximately 10 milliunits/g fresh wt. From the data of the Table it can be seen that for yeast transketolase, which does not contain fructose 1,6-diphosphatase, the ratio: activity of transketolase by method (IV)/activity of transketolase by method (V) was 5. The values of the same ratio for the partially purified heart transketolase and the untreated rat and rabbit heart extracts were 7 and 11, respectively. The increase in this ratio was attributed to the presence of significant levels of the contaminating enzymes particularly fructose 1,6-diphosphatase. A comparison was also made of the activities of transketolase measured by the 5 different methods in a crystalline preparation of yeast transketolase and a partially purified preparation of rat heart transketolase. The activity as determined by the method of BRUNS et al.[2] (method I) compared well with the activity determined by method (II) for yeast transketolase but was significantly greater for the less pure preparation of rat heart transketolase. Considerable variation about the mean value was also apparent for the colourimetric method[2]. The activities determined by the methods (III) and (IV) were found to be similar but approx. six-fold greater than that determined by method (V), consistent with previous observations[6] that hydroxypyruvate was a less efficient ketol donor for transketolase than was xylulose 5-phosphate.

Although method (V) (Table) does not express the maximum catalytic capacity of transketolase it is a convenient, reproducible and specific assay suitable for comparative studies and was found to be ideally suited for the comparative measurement of transketolase activity changes in heart extracts during hypertrophy. The choice of method (V) (Table) was further reinforced by the observations that neither hydroxypyruvate nor CO_2 resulted in the reduction of $NADP^+$ by enzymes of the heart extracts, and oxidation of NADPH did not occur.

Using this method we have found that the catalytic activity of transketolase decreased to 29% of its original activity 7 days after aortic constriction (Table) and that the activity only returned to the level of normal heart after 25 days. These results contradict the previous report of an increase of 60% in transketolase activity during hypertrophy[1] where the less specific assay method for transketolase was used.

We conclude that the values for the activity of transketolase in heart preparations as determined by colourimetric methods should be treated with caution and in situations where the rate of oxidation of NADH is relatively high the comparative activity of transketolase may be determined by the specific method described.

Zusammenfassung. Methodenvergleich zur Transketolase-Analyse in Rattenherzen. Die neue Methode benützt als Substrat Hydroxypyruvat und Erythrose-4-Phosphat und bestimmt das Verhältnis der Transketolaseaktivität an der Bildung von Fructose-6-Phosphat. Der Wechsel der Myocard-Transketolaseaktivität während Hypertrophie wird beschrieben.

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