with less contrast, such as biological specimens. The microradiogram (2500 volt) of a 5 μ thick section of a blood vessel wall is shown in Fig. 3. It is clear that the contrast is good, and so is the resolution. Figs. 5, 6 and 7 show microradiograms of microtome sections from rabbit epiphyseal cartilage, rabbit ear and pathological kidney respectively. Fig. 4 taken with a similar tube with 200 μ Be-window at 5 kV illustrates that the tube also can be used to study the distribution of calcium salts in a ground section of bone tissue. Another example of this type of application is Fig. 8 which shows calcifications in a kidney (5 μ thick section).

It was originally suggested that a small reference system should be radiographed simultaneously with the sample[2] when quantitative measurements have to be performed in the microradiogram. This procedure requires a relatively large homogeneous field of X-rays. The reference system can be made of thin nitrocellulose foils. By comparing the density of a biological structure with that of the steps in the reference system the weight of the structure can be computed[2]. There are certain difficulties in preparing a good reference system and also in determining the mass of each step[3,7]. Several methods have been proposed both for the preparation of the reference system and the measurement of the mass of each step[2,3,7]. The simple construction of the scaled off tube as compared with that of continuously pumped tubes enables us, however, to eliminate the necessity of a reference system. Instead of exposing only one specimen on each film, more, *e.g.* six, different samples can easily be registered on the same film. Each sample, however, is exposed differently, *i.e.* the time is varied with constant X-ray intensity causing different densities. The density of the X-ray image of the structure to be investigated and the density corresponding to the incident X-ray intensity are measured in each of the microradiograms. As all samples are registered on the same film it is possible from the measurement of the densities caused by the incident beam to construct the density-exposure curve for each individual photographic film, and no standard processing is necessary. From this density curve the X-ray transmission of any cytological structure in any of the six different samples can be determined. In order to convert X-ray transmission into weight per unit area, the nitrogen mass absorption coefficient for the radiation must be known, and this coefficient is determined for each voltage used by measuring the absorption of the X-rays in a certain distance of air.

Summarizing, the new tube described in this communication makes quantitative microradiography simple. The main advantages over old equipment for historadiography are: 1. No continuous pumping; 2. Small and compact construction; 3. Simple high voltage generator of small size; 4. No special reference system required if the procedure indicated above is followed; 5. High image resolution due to very small focal spot.

The tube is now being further tested for historadiographic work and most probably several modifications of the equipment and technical procedure will be introduced. The goal is to get an equipment as easy to use as an ordinary light microscope.

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GUANOSINE TRIPHOSPHATE, THE PRIMARY PRODUCT OF PHOSPHORYLATION COUPLED TO THE BREAKDOWN OF SUCCINYL COENZYME A*

by

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The enzyme system that catalyzes the phosphorylation of ADP** coupled to the breakdown of succinyl CoA consists of at least two enzymes[1] and an additional coenzyme[2]. The latter has been

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** The following abbreviations will be used: adenosine di-, and triphosphates, ADP and ATP;

isolated from yeast by adsorption on charcoal, followed by heavy metal precipitation and finally chromatography on Dowex-1 formate[3]. The absorption spectra of the isolated compound in acid and in alkali were identical with the spectra of guanosine and GMP-5'***. The base obtained by acid hydrolysis was indistinguishable from guanine in two paper chromatographic systems[4,5]. The compound contained ribose[6] and phosphate in the proportions shown in Table I. One of the two phosphate groups was labile in acid (1 *N* HCl, 100°, 15 min). Nucleotide pyrophosphatase§[7] liberated 1 mole of P_i from the compound per mole of base, the initial rate of hydrolysis being 27 times faster than that for GMP-5' under the same conditions. GMP-2' and GMP-3' were hydrolyzed even more slowly than GMP-5'. The cleavage product derived from nucleotide pyrophosphatase hydrolysis had the same R_F as GMP-5' in two solvent systems[5,8] both of which separated the three guanosine monophosphates. It may be concluded from the above data that the "phosphorylation coenzyme" is guanosine-5'-pyrophosphate (GDP)[3,9].

TABLE I

COMPOSITION OF COENZYME

	μmole per mg	*Molar ratio*
Guanosine (A_{260})	1.94	1.00
Ribose (Orcinol)	1.81	0.94
Phosphate (total)	3.59	1.85
Phosphate (labile)	1.74	0.90

The reaction catalyzed by enzyme A can be represented as shown in Equation (1).

$$\text{Succinyl CoA} + \text{GDP} + P_i \underset{}{\overset{\text{A, Mg}^{++}}{\rightleftharpoons}} \text{Succinate} + \text{CoA} + \text{GTP} \qquad (1)$$

Consistant with this formulation, the enzyme A-catalyzed disappearance of succinyl CoA (which is dependent on both P_i and GDP) is equivalent to the CoA liberated. When Reaction 1 is coupled to the KG dehydrogenase system where succinyl CoA is resynthesized (Reaction 2) as described

$$\text{KG} + \text{CoA} + \text{DPN}^+ \longrightarrow \text{Succinyl CoA} + \text{DPNH} + H^+ + CO_2 \qquad (2)$$

previously[2], the DPNH produced is equivalent to GDP added which forms the basis of the assay for GDP. GTP†[10] has been isolated from such a coupled reaction carried out in the presence of radioactive P_i. The identification of the GTP rests on the following properties: 1. spectra in acid and in base; 2. paper chromatography of the base released by acid hydrolysis; 3. presence of two acid labile phosphates per mole of base; 4. ^{32}P specific activity of the product was the same as that of the P_i (Table II). The ATP produced on incubating the isolated GTP with ADP, $MgCl_2$ and enzyme B

TABLE II

GTP SYNTHESIS

KG (15 μmoles), phosphate (36 μmoles, 91,000 CPM/μmole), $MgCl_2$ (40 μmoles), CoA (0.13 μmole), cysteine (8 μmoles), DPN (12 μmoles), GDP (2.2 μmoles), KGDH and enzyme A in 12 ml. Incubated 16 min at 25°. The GTP was obtained by adsorption on charcoal followed by precipitation of the barium salt. The *iso*butyric acid—ammonia system[8] was used for the separation of the nucleotides.

For ATP synthesis—GTP (0.7 μmole), $MgCl_2$ (2 μmoles), THAM buffer (5 μmoles), ADP (0.9 μmole) and enzyme B were incubated in 0.4 ml at pH 7.2 for 5 min at 30°.

	R_F	*CPM/μmole*
GTP	0.15	95,600
ATP	0.31	74,300

guanosine mono-, di- and tri-phosphates, GMP, GDP and GTP; inosine di- and tri-phosphates, IDP, ITP; di- and tri-phosphopyridine nucleotides, DPN and TPN; coenzyme A, CoA; α-ketoglutarate, KG and inorganic phosphate, P_i.

*** GMP-5', GMP-3' and GMP-2' were gifts from Dr. WALDO COHN.

§ Kindly supplied by Dr. T. SINGER.

† GTP has been simultaneously synthesized by STROMINGER from GDP and phosphopyruvate (reported at the Meetings of the American Society of Biological Chemists, see[9]).

was radioactive, the specific activity being only slightly less than that of the GTP. The ATP was identified by paper chromatography and by TPN reduction in the hexokinase-glucose-6-phosphate dehydrogenase system. GTP was essentially inactive with hexokinase under these conditions. Thus, enzyme B catalyzes the transphosphorylation between GTP and ADP (Equation 3) and may be designated GTP-ADP transphosphorylase.

$$GTP + ADP \overset{Mg^{++}}{\rightleftharpoons} GDP + ATP \qquad (3)$$

Of a series of nucleotides that have been tested, only IDP will replace GDP in the primary phosphorylation reaction. From the constant ratios of activities with GDP and IDP (slightly above 2) at different stages of purification of enzyme A, prepared from beef heart muscle as well as from pork kidney, it appears probable that the same enzyme catalyzes Reactions 1 and 4. For the synthesis of succinyl CoA from ITP only enzyme A (but not enzyme B) is required (Table III). That the

$$\text{Succinyl CoA} + IDP + P_i \overset{A,\ Mg^{++}}{\rightleftharpoons} \text{Succinate} + CoA + ITP \qquad (4)$$

GTP-ADP transphosphorylase preparation catalyzes also the ITP-ADP transphosphorylation[8,11] is shown by experiments coupled to the ketoglutaric dehydrogenase system (compare 2).

TABLE III

SUCCINYL CoA SYNTHESIS WITH ITP

The complete system consisted of CoASH (0.13 μmole), ITP (0.82 μmole), succinate (5 μmoles), THAM (5 μmoles), $MgCl_2$ (1 μmole), KBH_4 (1 μmole) and enzyme A (30 γ) in 0.3 ml at pH 7.5. Incubated for 5′ at 30°. The practically complete utilization of CoA suggests a method for CoA assay.

	μmole Δ-SH
Complete system	— 0.12
No ITP	0.00
No succinate	+ 0.01
No ITP, no succinate	+ 0.01
No succinate, no enzyme	0.00

It may be noted that, under the conditions described in Table III, there is no decrease in –SH without added succinate (line 3). We have also observed that both GDP and P_i are required for the breakdown of succinyl CoA catalyzed by enzyme A. These last two observations indicate that *stoichiometric* amounts of an intermediate involving P_i and CoA (*e.g.*, phosphoryl CoA) are not formed although formation of catalytic amounts is not excluded.

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