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[1] Smuckler, E. A., Iseri, O. A., and Benditt, E. P., *J. Exp. Med.*, **116**, 55 (1962).
[2] Rees, K. R., Sinha, K. P., and Spector, W. G., *J. Path. Bact.*, **81**, 107 (1961).
[3] Flores, G., Rosado, A., Torres, J., and Soberon, G., *Amer. J. Physiol.*, **203**, 43 (1962).
[4] Reichard, H., *J. Lab. Clin. Med.*, **53**, 417 (1959).
[5] Bengmark, S., and Olsson, R., *Proc. Soc. Exp. Biol. Med.*, **109**, 258 (1962).
[6] Cohen, P. P., and Sallach, H. J., *Metabolic Pathways*, 2, 1 (Academic Press, New York, 1962).
[7] Bassi, M., *Exp. Cell. Res.*, **20**, 313 (1960).
[8] Recknagel, R. O., and Lombardi, B., *J. Biol. Chem.*, **236**, 564 (1961).
[9] Recknagel, R. O., and Litteria, M., *Amer. J. Pathol.*, **36**, 521 (1960).
[10] Rossi, F., and Zatti, M., *Brit. J. Exp. Path.* (in the press).
[11] Brown, jun., G. W., and Cohen, P. P., *J. Biol. Chem.*, **234**, 1769 (1959).

Visualization of Different Diphospho- and Triphospho-pyridine Nucleotide Dehydrogenase Isozymes on an Agar-gel Plate

We have been looking for a quick and relatively cheap method of screening dehydrogenase isozymes, after electrophoresis. For this purpose, we chose agar gel as a support. We recognize that there is a greater diffusion on agar gel than on starch gel, and also that there is some difficulty in slicing the gel before detecting the isoenzymes. Nevertheless, we prefer agar gel because: (1) it allows a relatively quick electrophoretic partition; (2) it is transparent when dry; (3) it allows the use of the Grabar and Williams technique of immunoelectrophoresis[1].

For this medium, we developed a convenient tetrazolium method which: (1) limits the diffusion during the reaction period: (2) requires a minimal quantity of reaction components, some of them being very expensive; (3) can be used rapidly for different purposes on the same plate.

So far as we know, the method described here seems quicker, cheaper and more versatile than some other isozyme methods using tetrazolium salts[2,3].

The electrophoresis was carried out in a 1 per cent agar gel ('Agar Noble', Difco), in 0·05 M phosphate buffer at *p*H 7 for a good partition of slow components, or in *tris* buffer 0·05 M when phosphate ions are to be avoided. The gel was poured on photographic glass plates (13 × 18 cm for 4 samples, or 18 × 24 cm for 8 samples). Each sample was inserted in a 20 × 3 mm slit cut out of the agar gel with a special punch. The preparation was kept for 105 min in a field of 6 V/cm in the cold (+ 4°). The plate was then brought to laboratory temperature. It was covered with a wooden board which has been wetted before application so that it will stick to the agar gel. This little board outlined 4 or 8, 25 × 150 mm slits which lay over the fractionated samples. In each slit of the board was poured a solution containing the buffer, some substrate, coenzyme, oxidation inhibitor electron transporter (phenazine methosulphate) and tetrazolium salt, in 1 per cent agar gel.

For the detection of lactic dehydrogenase in plasma, 0·1 ml. of plasma was mixed with 0·1 ml. of 2 per cent agar gel. During the time the electrophoresis is being run in phosphate buffer at *p*H 7, the following solutions was prepared:

(*a*) Aliquots of a stock solution of the following compositions, prepared beforehand, and stored at 35° C in 5 ml. vials, were taken from the freezer: sodium lactate, 30 mg; diphosphopyridine nucleotide (Sigma), 2 mg; sodium azide, 10 mg; 3(4,5 dimethyl thiazolyl 1,2)2,5 diphenyl tetrazolium bromide (ATT: Mann), 2 mg (dissolved in 0·2 ml. acetone); *tris* buffer (0·05 M, *p*M 8·2), 2 ml.

The solution was transferred to a hæmolysis tube and warmed to 42° C. To this was added 2 ml. of 2 per cent agar gel in water, then 0·2–0·5 mg of sodium phenazine methosulphate (Sigma). After quick mixture, it was poured in one of the slits and immediately covered, to protect the complex from light.

After incubation for 1 h at 37° C in a damp atmosphere or 2 h at laboratory temperature, the isozyme pattern was very apparent. If desired it could be covered with a thin layer of 1 per cent agar gel in order to obtain a flat surface. Afterwards, the plate was fixed for 2 h in 2 per cent acetic acid. The preparation was then covered with filter paper and dried at 37° C.

The plate with the dried film could be kept indefinitely in the dark, and filed as a laboratory record.

We applied this system to visualize the isozymes of lactic acid dehydrogenase of normal and pathological plasma, as well as the isozymes of lactic, malic, isocitric, glutamic, β-hydroxybutyric and glucose-6-phosphoric acid dehydrogenases in rat liver homogenates. In addition, we applied our system after plasma immunoelectrophoresis. We are now examining the coloured precipitation lines obtained to determine whether they are enzymatically active.

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[1] Grabar, P., and Williams, C. A., *Biochim. Biophys. Acta*, **17**, 65 (1955).
[2] Vesell, E. S., *Ann. N.Y. Acad. Sci.*, **94**, 877 (1961).
[3] Markert, C. L., and Appella, E., *Ann. N.Y. Acad. Sci.*, **94**, 678 (1961).

PHYSIOLOGY

Uterine Relaxant Properties of Bradykinin *in vitro*

At the present time, there is no effective means of blocking uterine contractions in order to prevent premature labour. A drug which would block uterine contractions may reduce the incidence of prematurity. Of all infant deaths occurring at the New York Hospital in 1961, 72·5 per cent were prematurely born.

Bradykinin was synthesized by Boissonnas, Guttmann and Jaquenoud[1] and was shown to be a nonapeptide. Elliot, Horton and Lewis[2] demonstrated the great sensitivity of the isolated rat uterus to bradykinin as indicated by increased contractility. Because of this constrictor response in the rat, Berde and Saameli[3] investigated the effect of bradykinin on the human uterus *in situ*. Intravenous injections of 2 μg/kg were without any effect on the human uterus. These authors concluded that bradykinin had no constrictor activity on the human uterus. Because of its known relaxant effect on smooth muscle, bradykinin in various concentrations was applied to human uterine muscle. Strips were placed in an organ bath of 5 c.c. capacity containing oxygenated mammalian Krebs's solution[4] which was kept at constant temperature and *p*H.

Bradykinin was applied 112 times to 32 non-pregnant uteri and 52 times to 13 pregnant uteri at the time of section. The effect on tonus, frequency and amplitude maximum was recorded after a pattern of spontaneous or induced contractions was established. A reduction in at

Table 1. Effect of Bradykinin on the Human Uterus *in vitro*

	Concentration of bradykinin (μg/ml.)						
	0·2	0·4	0·6	0·8	1·0	1·2	1·4
Non-pregnant: Incidence of response	11/20 55%	27/32 84·5%	17/18 94·5%	29/30 96·5%	12/12 100%		
Pregnant: Incidence of response	6/10 60%	12/12 100%	3/4 75%	13/13 100%	6/6 100%	4/5 80%	2/2 100%