Effects of increasing Mg^{++} ion concentration on the PKU monitoring assay

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Summary. Inaccuracies in a commonly used bacterial inhibition assay for blood phenylalanine levels arise when the Mg^{++} ion concentration in the assay medium is increased. This has practical implications in the diagnosis and management of phenylketonuria.

Gram-negative bacterial genera have been differentiated according to inhibition reactions to β-2-thienylalanine (β-2-t) in a plate assay[2]. When the defined medium[3] used in the inhibition assay was compared with the medium of Davis and Mingioli[4], it was found that inhibition was abolished for all genera in the latter medium due to increased Mg^{++} ion concentration[5].

The effects of increasing Mg^{++} ion concentration were then compared in the Guthrie phenylketonuria (PKU) screening assay[6], a test also based upon bacterial inhibition by β-2-t. Random blood samples from 112 infants were tested. 5 duplicate plates, with concentrations of 0, 0.01, 0.02, 0.05 and 0.1 g/l $MgSO_4$, respectively, were used to test identical series of specimens. In plates containing no magnesium, all samples were negative for increased blood phenylalanine levels (≤ 4 mg/100 ml). In the test plate containing 0.05 g/l $MgSO_4$, 4 of the samples were positive (> 4 mg/100 ml)[5]. Commercial 'PKU test agar' (BBL) contains 0.05 g/l $MgSO_4$. For a sample to be read as positive, growth of the *Bacillus subtilis* test strain must be equal to or greater than that surrounding a control disk impregnated with blood containing 4 mg/100 ml phenylalanine.

The observations of the effects of increasing Mg^{++} ion have now been extended to the PKU monitoring assay. The effectiveness of dietary therapy for reducing blood phenylalanine levels in PKU patients is monitored through periodic blood samples, which are measured in the Guthrie assay[6]. Although normal human levels of phenylalanine are 0.8–1.2 mg/100 ml[7], PKU blood samples are considered acceptable if in the range of 6–12 mg/100 ml in our clinic.

55 monitoring samples from PKU patients were tested in 5 duplicate Guthrie assay plates to which 0, 0.01, 0.02, 0.05, and 0.1 g/l $MgSO_4$ were added, respectively. Of 28 samples which read 6 mg/100 ml phenylalanine or less in the control plate (0 g/l $MgSO_4$), 3 samples gave readings of greater than 4 mg/100 ml on plates containing 0.05 and 0.1 g/l $MgSO_4$, while giving readings of less than 4 mg/100 ml on the control plate. Increasing of $MgSO_4$ to concentrations of 0.05 and 0.1 g/l resulted in decreasing differences between adjacent control samples in a series containing 0, 2, 4, 6, 8, 10, 12 and 20 mg/100 ml phenylalanine. As test sample phenylalanine concentrations were determined by comparison with the control series, determinations were made more difficult under conditions of high Mg^{++} ion, than in the plates containing 0–0.02 g/l $MgSO_4$ (figure).

Inaccurate readings in the Guthrie assay can lead to incorrect treatment regimens. In the screening assay, positive samples should be checked by an additional method[6]. False positives, if not discounted by determination of phenylalanine levels by amino acid analysis or by retesting, could result in initiation of treatment for PKU[5]. In the monitoring assay, PKU patients with near-maximum acceptable levels (10–12 mg/100 ml phenylalanine) could be

Plate A

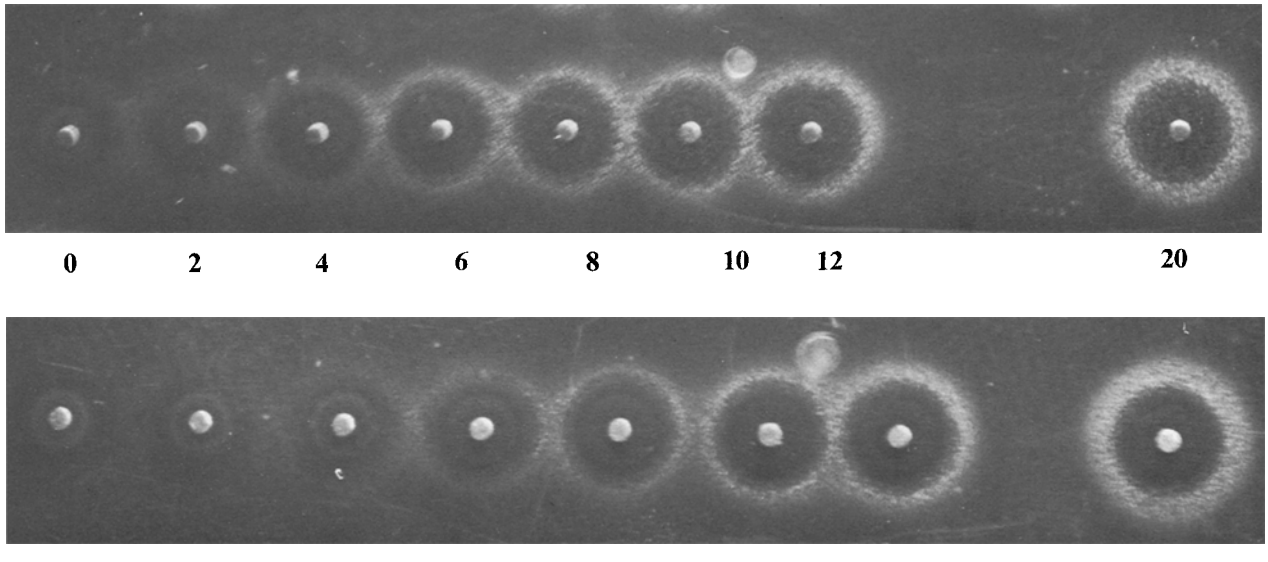

Plate B

Guthrie assay[6] for PKU testing. *Bacillus subtilis* spores and β-2-thienylalanine are included in the medium. The control series of blood-impregnated filter paper disks contains increasing phenylalanine concentrations (from left, 0, 2, 4, 6, 8, 10, 12, and 20 mg/100 ml). Phenylalanine reverses the inhibition of spore germination by β-2-t. Plate A contains 0.05 g/l $MgSO_4$, the amount present in commercial PKU test agar, while plate B contains 0 g/l $MgSO_4$. Adjacent samples are more difficult to differentiate in plate A than in plate B, and the increased 'background' growth makes comparisons of test samples with the control series more difficult to read. Plates containing 0.01 g/l $MgSO_4$ read similarly to plate B.

unnecessarily restricted in protein intake, if inaccurate high readings are obtained. Blood specimens from PKU patients containing less than 4 mg/100 ml phenylalanine, which are incorrectly assessed as greater than 4 mg/100 ml, could result in dangerous overtreatment, which could lead to decreased intelligence through phenylalanine and protein deficiency[8].

It is suggested that the PKU screening and monitoring assays be modified by reducing the concentration of $MgSO_4$ in the medium to 0.01 g/l or less.

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Complete development of human hookworm, *Ancylostoma duodenale* (Dubini, 1843) in infant rabbits

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Summary. Establishment of a patent infection of *Ancylostoma duodenale* in the laboratory host, infant rabbit, is successfully achieved.

Hookworm infection is one of the major cosmopolitan and pathogenic diseases of mankind, especially in the tropics[2,3]. The development of a laboratory model for various experimental studies for hookworm has been, and remained, a vital concern in the control of the disease. The review of literature suggests that human hookworm, *Ancylostoma duodenale*, fails to undergo any development or develops upto the 4th stage in mice[4,5]. Complete development is possible in pups[6], but these large animals are found unsuitable for various laboratory investigations. The importance of the laboratory animal-adapted strain of *A. duodenale* has long been realized, especially for the chemotherapeutic and other host-parasite studies. The object of our investigation was to determine whether neonatal rabbits could be a suitable host for *A. duodenale*. The successful results, for the first time, present evidence in this communication.

Materials and methods. The larvae were obtained from 10–12-day-old culture prepared from human patients who were naturally infected with hookworm[7]. 9 infant rabbits, 4–6-day-old, average b.wt 55 g, were infected with infective larvae by mouth (buccal pouch) route, each animal received a single dose of 3000–5000 larvae in 0.02 ml of saline suspension. After infection, animals were replaced with their respective mothers. Animals were necropsied or autopsied on various days of infection. Developing larvae were recovered from the lungs and liver by pepsin digestion process[4]. Parasites were collected from the gastrointestinal tract and they were washed, counted, fixed in hot AFA

Table 1. Recovery of *Ancylostoma duodenale* larvae/worm from the lungs, liver and small intestine of rabbits after oral administration of larvae

Generation	Number of animals	Dose of infection	Duration of infection (days)	Number of animals killed/died	Faecal examination egg	Faecal examination Larval yield on coproculture	Larvae/worms recovery Lungs	Liver	Small intestine	Total
1	9	3000–5000	3	1 K			52	2	–	54
			5	1 K			10	–	–	10
			7	1 D			–	–	–	–
			13	1 D			–	–	–	–
			21	2 D*			–	–	3 (2♂ 1♀)	3
			30	1 K			–	–	8 (4♂ 4♀)	8
			47		8000	+				
			60	1 K		–	–	–	–	–
			90	1 K		–	–	–	–	–
2	5	5000	2	1 D			+**	–	–	–
			3	1 D			+**	–	–	–
			20	1 K			–	–	–	–
			36		350	+				
			38		3600	+				
			40		3800	+				
			42		2400	+				
			45		400	+				
			47		200	+				
			50	2 K	–	–			–	–

*Out of 2 one was positive. **Larval number not counted.