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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2 K.J. Brown and D.R. Lines, Can. J. Microbiol. *22*, 1673 (1976).
3 A.L. Demain, J. Bact. *75*, 517 (1958).
4 B.D. Davis and E.S. Mingioli, J. Bact. *60*, 17 (1950).
5 K.J. Brown and D.R. Lines, Aust. J. exp. Biol. med. Sci. *56*, 507 (1978).
6 R. Guthrie and A. Susi, Pediatrics *32*, 338 (1963).
7 P.L. Altman and D.S. Dittmer, ed., Biology Data Book, vol. 3. Federation of American Societies for Experimental Biology, Bethesda, Md. 1974.
8 M.E. Blaskovics and T.L. Nelson, Calif. Med. *115*, 42 (1971).

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.

Table 2. Average dimensions in mm of developing stage of *A. duodenale* in infant rabbits

Stage of development	3rd stage before infection	Initial 3rd stage (3)*	Advanced 3rd stage (5)*	Immature adult stage (21)* Male	Female	Adult stage (30)* Male	Female
Total length	0.450	0.550	0.590	6.500	6.700	5.93	7.08
Total width	0.020	0.020	0.020	0.275	0.300	0.250	0.250
Bucal capsule (Length and width)				0.017× 0.013	0.020× 0.015	0.017× 0.013	0.020× 0.015
Oesophagus length	0.120	0.185	0.190	0.825	0.850	0.900	1.070
Tail length/bursa				0.023	0.025	0.025	0.025
Spicule length				1.132	-	0.135	-
Gubernaculum length				0.032	-	0.032	-

*Number in brackets: days after infection.

solution (50% alcohol, 100 parts + formaline, 6.5 parts + glacial acetic acid, 2.5 parts) for measurement and identification. All the preserved developing stages were carefully identified and they showed all the characteristic features of *A. duodenale* only[8,9].

Infective larvae obtained from 10–12-day-old faecal culture prepared from pooled faeces of infected rabbits were passaged to another group of 5 infant rabbits of same age and b.wt. Egg production in the animals was determined by collecting pooled faeces by Stoll's technique[10].

Results. During the first 3 and 5 days infection, initial and advanced 3rd stage larvae were recovered mostly from the lungs and some from the liver. The larvae showed increase in size with sharp needle-like caudal end. On days 21 and 30, immature and mature adults were recovered, respectively, from the intestinal tract (tables 1 and 2). These worms showed typical ancylostome's buccal capsule provided with characteristic 2 pairs of hook-like teeth on the ventral surface and bursa with 2 separate spicules in male (figures 1 and 2). The fertile eggs in faeces first appeared on day 47, yielded 160,000 motile larvae when approximately 20 g pellets from the infected rabbits were cultured. Rabbits were found negative for worms on 60th and 90th days post infection.

In generation 2, out of 5 rabbits, 2 rabbits died on days 2 and 3 and one rabbit was killed on the 20th day post infection. The larvae were seen in lungs of the first 2 animals but no worm was recovered from the intestinal tract of the 3rd one. The eggs first appeared in the faeces of 2 rabbits on the 36th day and they developed into infective larvae after 8–10 days when cultured. The egg production per g rose to 3600 on the 38th day, reached a maximum of 3800 on the 40th day, declined to 2400 on the 42nd day and subsequently decreased to below 500 on days 45 and 47. The eggs finally disappeared on the 50th day post exposure. On necropsy, these remaining 2 rabbits did not show any worm recovery from the intestine on the 50th day and no larvae were recovered on faecal culture as well.

Discussion. The present investigation demonstrates that infant rabbits are susceptible to infection with *A. duodenale.* The larvae reach the adult stage to sexual maturity within 4 weeks, to the oviposition within 5 weeks and remain at that stage until seven weeks post exposure. This study also shows that adult worms are retained in the host's intestine for a period of 47 days. This, thereby, indicates a short duration of about 7 weeks as compared to another human hookworm species, *Necator americanus,* in the same host for about a period of 150 days[11]. On the other hand, *Ancylostoma ceylanicum* fails to undergo any development in infant as well as young rabbits[12]. However, *A. duodenale* shows an incomplete development in the laboratory mouse[5].

The present investigation suggests the neonatal rabbit as a valuable tool for further research on ancylostomiasis caused by *A. duodenale.*

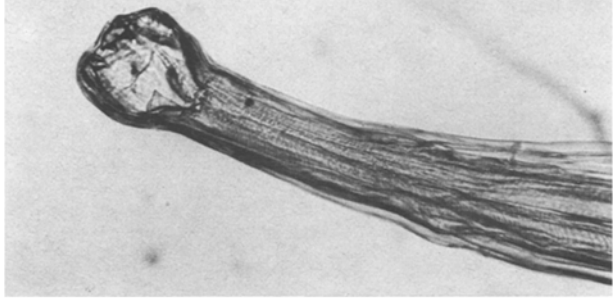

Fig. 1 and 2. Photomicrographs of head (Female) and copulatory bursa (Male) of mature *Ancylostoma duodenale* recovered from infant rabbits. Fig. 1. Head of a female worm. Fig. 2. Bursa of a male worm.

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2 M.R. Stoll, J. Parasit. *33,* 1 (1947).
3 R.R. Arora, R. Biswas and K.K. Mathur, J. Com. Dis. *8,* 66 (1976).
4 C.T. Soh, J. Parasit. *44,* 515 (1958).
5 S. Chattervati, M.K. Gupta and V.K. Vinayak, Indian J. Parasit. *2,* 65 (1978).
6 M. Nagahana and Y. Yoshida, J. Parasit. *51* (sec. 2), 52 (1965).
7 H.G. Sen, U.N. Joshi and D. Seth, Trans. R. Soc. trop. Med. Hyg. *59,* 684 (1965).
8 Y. Yoshida, J. Parasit. *52,* 122 (1966).
9 P. Sood, R.A. Bhujuvala and Om Prakash, Ind. J. med. Res. *60,* 1010 (1972).
10 N.R. Stoll, Parasitology, protozoology and helminthology in relation to clinical medicine, 5th ed., p. 183. Sree Saraswaty Press Limited, Calcutta 1964.
11 M.K. Bhopale, S. Menon and D.M. Renapurkar, Bull. Haff. Inst. *5* (1), 38 (1977).
12 M.K. Bhopale, S. Menon and D.M. Renapurkar, Bull. Haff. Inst. *6,* 68 (1978).