

# A NEW SCREENING TEST FOR CATECHOLAMINES PRESENCE IN THE URINE OF PATIENTS

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*The time required for the disappearance of the white spots produced by an injection of catecholamines containing urine sample into Siamese fighting fish was used as a screening test as an early diagnosis of patients having catecholamines secreting tumour. The urinary catecholamines test using the fish has previously been reported by Songkittiguna.<sup>(1)</sup> The time required for the disappearance of the white spots produced by the injection into the fish with the morning urine samples obtained from the patients under 12 years (n=32) was not statistically different from the patients over 12 years (n=16) ( $p>0.05$ ). In addition, the white spots produced by both the patients over 12 years and the all age (ten days to 70 years) did not differ significantly from each other ( $p>0.05$ ) (n=16 and 48, respectively). Interestingly, the disappearance time of the white spots produced by the injection of the urine samples from a certain group of patients having catecholamine secreting tumour (under 12 years, (n=12)), were markedly longer and highly significant different from the previous group ( $p<0.001$ ).*

## INTRODUCTION

Neuroblastoma of the medulla is the most common infantile adrenal neoplasm. About thirty and eighty percents of the patients are diagnosed within less than one year and five years, respectively. Less than 5 percents of the patients are diagnosed after over 15 years of age.<sup>(2)</sup> Next to retinoblastoma, neuroblastoma is the most common congenital cancer. Like many other cancers, neuroblastoma can be

successfully treated if the diagnosis is early enough. Although HPLC is an established method for the detection of vanillylmandelic acid (VMA) and homovanillic acid (HVA), the Newcastle team found that it is a time consuming method and is too slow to screen 200 samples per day. Therefore, this screening test was developed in order to overcome the complexity of HPLC method by using a very simple methodology.

## MATERIALS AND METHODS

### A. An animal preparation

The inbred-male Siamese fighting fish of 6-8 months in age were used. Their displaying of fighting signs indicated a healthy condition and ready for the test which was carried out in an air-conditioning laboratory (24-26°C). One hour before experiment, the fish was transferred from an animal house into the laboratory in a 100 ml glass beaker containing 60 ml of distilled water. When the fish saw each other through the transparent glass beaker; they would show visible signs of fighting such as stretching of fins and tails which were red and blue colours throughout the body.

### B. The preparation of urine samples

All of the lying-in patients suspected to have catecholamines secreting tumors were chosen as subjects. Approximately 5 ml of the fresh morning urine sample collected before midday from the in-patients and a group of patients suspected to have the catecholamines secreting tumour was kept in a plastic container in the presence of approximately 25 mg/l ascorbic acid. After collection, the urine container was immersed immediately into the ice and kept there until studied.

### C. Testing procedure

Anaesthetization and intramuscular injection of the urine sample to the fish

The fish were anaesthetized by hypothermia. Using a plastic-hub luer type hypodermic syringe and needle (guage 26, 1/2") with

microverneer, 50 ul of the urine was injected into a muscle under the dorsal fin of the fish.<sup>(3)</sup> After stoppage of bleeding at the injected site for a few seconds, the fish were then returned to their original containers. Timing was commenced immediately after the injection. The time required for the disappearance of the white spot at the injection site was then recorded; the timing ended when the white spot had returned to original colours of the fish.

## RESULTS

The results of the time required for the disappearance of the white spot are shown in Table 1. and Fig. 1. In 32 young patients without catecholamine secreting tumor (age of 10 days to 12 years) the disappearance time of the white spot was  $41.56 \pm 5.14$  min. The urine samples from the other group of patients (age of 13 to 70 years) showed very similar result,  $39.58 \pm 5.39$  min. While the average time from all ages of 48 patients was  $38.57 \pm 5.11$  min. Those results were counted as normal values. In 12 patients suspected to have catecholamine secreting tumor (under 13 years of age) the time was  $178.95 \pm 8.19$  min; the other patient (age > 15 years), it was 232.5 min. The average time of this two groups of patient altogether was  $187.15 \pm 9.11$  min. This markedly prolonged time for the disappearance of white spot was counted as abnormal urine. The white spots produced by the injection of the urine sample obtained from the patients with catecholamine-secreting tumours, neuroblastoma and phaeochromocytoma, were shown in Fig. 3 and 4 respectively. Fig. 2 shows the white spot produced by injection of the urine

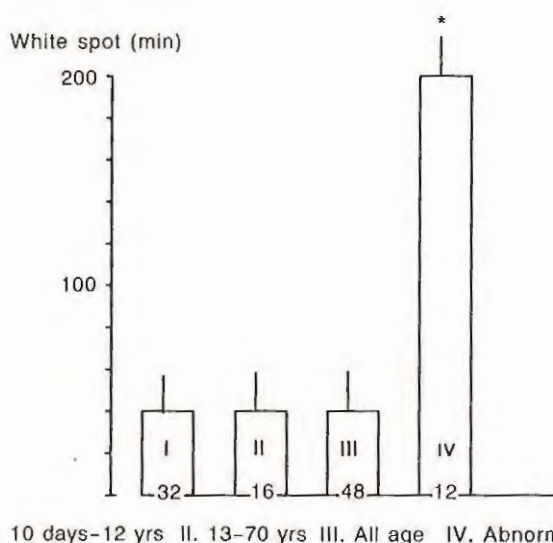
**Table 1** The time required for the disappearance of the white spot caused by the interaction of the patients' urine with the fish.

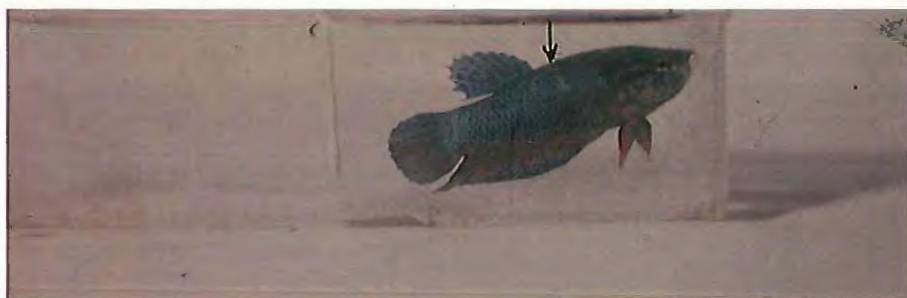
## A FISH TEST

URINE	DISAPPEARANCE OF WHITE SPOT * $\bar{X} \pm S.D.$ (min)	n
Urine samples from patients without catecholamine secreting tumours Normal urine (50 ul)		
Age, 10 days - 12 years	41.56 $\pm$ 5.14	32
Age, 13 - 70 years	39.58 $\pm$ 5.39	16
Total	38.57 $\pm$ 5.11	48
Urine samples from patients suspected to have catecholamines. Abnormal Urine (50 ul)		
Neuroblastomas	178.95 $\pm$ 8.19 *	12
Pheochromocytomas	232.50	1
Total	187.15 $\pm$ 9.11	13

\* The results were expressed as mean  $\pm$  standard deviation.

n is the number of patients.

**Fig. 1** The time of disappearance of the white spots produced by intramuscular injection of 50 ul of urine. The numbers at the base of histograms are the number of the patients. Asterisk (\*) is significant difference from I, II and III ( $p < 0.001$ ) unpaired Student's t-test.



**Fig. 2** A small white spot produced by an injection of 50 ul urine sample intramuscularly.  
(A patient with Wilm's tumour)



**Fig. 3** A large white spot produced by an injection of 50 ul urine sample intermuscularly.  
(A patient with neuroblastoma)



**Fig. 4** A large white spot with generalized pallor produced by an injection of 50 ul urine sample intramuscularly.  
(A patient with bilateral adrenal pheochromocytoma)



from a patient with non-catecholamine secreting tumour (Wilm's tumour).

## DISCUSSION

Since neuroblastomas are one of the deadly-disease in childhood; the early the diagnosis, the good prognosis will be made and the life-saving could be accomplished. The catecholamine-secreting tumours have been reported since early nineteen centuries.<sup>(4)</sup> The adrenal gland is a major source of plasma and urine catecholamines; the amount of individual catecholamine and their distribution may be useful in the interpretation of the different kinds of tumours.<sup>(5)</sup> In comparison to body weight, the adrenal glands are large at birth, weighing 2 to 4 grams each, or  $8.2 \pm 3.4$  grams together. At 10 to 15 years of age, the normal aggregate weight 8.5 grams in boy and 7.5 grams in girls. Whereas, normal adult adrenal glands removed surgically weigh  $4.8 \pm 0.89$  grams in man and  $4.1 \pm 0.8$  grams in woman. After sudden death, the aggregate adrenal weight is  $9.2 \pm 1.8$  grams in the United States. In autopsies in other situations the normal range is 12 to 16 grams, or 0.21 to 0.26 gram/kg body weight.<sup>(2)</sup> It could be seen that the adrenal glands at any ages in both sexes are about the same size.

As it was previously reported that the white spot produced by injection of the urine samples was due to the presence of catecholamines. Time required to finish the reaction of urinary catecholamines in the fish model (the disappearance of the white spot) does not, therefore, differ significantly between the patients under 12 years of age and the

patient under 70 years of age. The results also reflect the function of the adrenal glands which are well-known as a major source of urinary catecholamines. In 12 patients under 12 years of age and 1 patient of 16 years who were suspected to carry catecholamine secreting tumour, the disappearance time of the white spot was markedly delayed. The final diagnosis of these 12 patients was neuroblastoma and that of the 16-year-old patient was bilateral adrenal phaeochromocytoma. The 2 patients out of 12, showed a false negative result which was probably due to the low level of free catecholamines in the large mass of the tumours. The catecholamines appeared to be metabolized inside the tumour masses before entering the circulation and before excreting into the urine.<sup>(6)</sup> Furthermore, in patients having brief and infrequent paroxysms with symptom free intervals, confirmation of the diagnosis by a single collection of the urine sample may be insufficient.<sup>(7)</sup> These might cause the fish method to yield a false negative result; to overcome this obstacle, the collection of urine sample should be repeated during the symptom period. Therefore, the fish method could help diagnosis of the catecholamine-secreting tumours: neuroblastoma and phaeochromocytoma. In addition, Broadhurst and Briley<sup>(8)</sup> have made a conclusion that the assay of catecholamines provides rapid indication of the effect of drugs on neuronal activity and the measurement of in vitro and in vivo neurotransmitter releases could greatly enhance our understanding of neuronal systems and their modulation in vivo. Moreover, Rand<sup>(9)</sup> mentioned that bioassay is useful

for measuring the amounts of catecholamines in the pharmacological preparations or in tissue extracts and usually has a higher sensitivity than the conventional chemical or physical methods. The results of the present study are also in accord with the previous report by the author.<sup>(1)</sup> Therefore, the detection of naturally occurring neurotransmitters, the catecholamines, is worth studying using Siamese fighting fish with a view to screen the normal patient from the patient with high content of catecholamines in urine.

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