(tissue culture infective doses) for the adenoviruses to 10^7 TCD_{50} for the Coxsackie B viruses.

Several strains of Coxsackie virus Type B which had been isolated from human stool specimens in monkey kidney tissue cultures were also isolated in pig kidney cultures from the same specimens. On the other hand, poliomyelitis Types I, II and III viruses which had been isolated in monkey kidney cultures from similar specimens could not be isolated in pig kidney cultures.

Summary. 1) Susceptibility of pig kidney tissue cultures to the following viruses has been described: Coxsackie A and B, Echo, poliomyelitis, adenoviruses, and virus B. 2) Susceptibility of these cultures to Coxsackie group B viruses, virus B and the adenovirus group has been established. From the results it is apparent that pig kidney cultures may be utilized as an additional tool in the diagnostic differentiation between Coxsackie Group B viruses and the Echo virus group.

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Received July 10, 1957. P.S.E.B.M., 1957, v96.

Some Factors Affecting Differential Excretion of D-Phenylalanine in Man.* (23467)

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Marked variability between individuals in urinary excretion and utilization of D-phenylalanine by man has been noted by several investigators(1,2). It has also been recently observed through limited twin studies that some of this variability may have a genetic basis(2). Since the physiological basis of this differential excretion of D-phenylalanine could be quite complex, we felt it would be worth while to investigate various physiological aspects of this problem before going on with further genetic studies. Therefore, the present study of the role of the following physiological variables in the differential excretion of D-phenylalanine was undertaken: (1) absorption of D-phenylalanine into the blood, (2) metabolism of D-phenylalanine, and (3) renal excretion of D-phenylalanine.

* This work was supported by a grant from Natl. Science Fn.

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Methods and materials. The subjects were normal, healthy adults and were tested after an overnight fast. At this time, blood and urine samples were obtained and the subjects were then given orally 0.5 g of D-phenylalanine (Mann Laboratories) dissolved in 200 ml of water. Blood was taken from the anticubital vein into heparinized tubes and the plasma drawn off. The plasma and urine samples were then stored at −10°C until ready for analysis. On 2 of the 4 occasions where experiments were repeated on the same individuals, 1.0 g of D-phenylalanine was given instead of 0.5 g for the second trial. One hour after feeding, blood samples were again taken and the urine flow for the one hour test period collected. L-phenylalanine determinations were carried out on all plasma and urine samples by a modification(3) of the Udenfriend and Cooper decarboxylase method(4). D-phenylalanine was estimated by the difference between L-phenylalanine determinations and paper chromatographic estimations of DL-phenylalanine on the second blood and urine samples. Phenylpyruvic acid determinations

TABLE I. Summary of Data on Plasma and Urine Phenylalanine (Phe) and Urine Phenylpyruvic Acid (PPA) One Hour after Oral Administration of 0.5 g of D-phenylalanine.

Subject and sex	Age (yr)	Wt (kg)	Plasma, mg/100 ml: L-Phe Fasting	L-Phe Post feeding	D-Phe	Urine (mg/hr) D-Phe	PPA	Renal clearance† D-Phe
1 ♀	20	54.4	1.4	1.3	1.7	59.7	15.4	58.6
2 ♀	20	65.8			.7	27.4	1.4	64.3
2 ♀*			1.0	1.3	1.6	98.6	8.5	102.5
3 ♀	30	63.5			.8	52.1	9.0	108.8
3 ♀*			1.3	1.4	2.4	99.0	24.4	68.3
4 ♀	19	54.9	1.3	1.2	.7	7.1	0	15.4
5 ♀	20	58.5			.9	23.4	2.1	43.3
6 ♂	33	80.3	1.1	1.0	1.2	18.2	.5	26.2
6 ♂						38.1	0	
7 ♂	34	63.5	1.4	1.4	1.0	35.7	11.2	59.5
7 ♂						57.9	7.0	
8 ♂	33	72.7	1.2	1.2	1.0	33.3	0	55.0
9 ♂	33	68.0	1.0	1.0	1.5	40.8	.5	46.1
10 ♂	46	65.5	1.2	1.3	1.2	37.4	9.3	52.5

* Fed 1.0 g D-phenylalanine.

† $\frac{\text{Urinary D-Phe (mg/min.)}}{\text{Plasma D-Phe (mg/ml)}}$.

were carried out on urine samples according to a method by Armstrong(5).

Results. In Table I are presented the pertinent data on the subjects tested and the results of the analyses of their blood and urine samples.

As can be seen, there is considerable variation between individuals in the urinary excretion of D-phenylalanine (the range being over 3 fold), and no apparent sex difference. In the case of individuals 6 and 7, experiments with the same test dose were repeated on separate occasions. Considerable variation exists between different trials on the same individual, but the relative and apparently absolute difference between these two subjects is constant. Clearly some of the apparent differences between individuals in the excretion of D-phenylalanine must be ascribed to individual daily variability. However, a considerable portion remains which may be due to individual physiological differences.

We shall consider first the possibility of differential absorption of D-phenylalanine into the blood as a possible physiological explanation of these individual differences. An estimate of this factor can be obtained from column 6 which gives the plasma level of D-phenylalanine one hour after the ingestion of the substrate. The range of variation is approximately 2-fold and there appears to be a general relationship between the plasma level of D-phenylalanine and the amount excreted in the urine. Statistical evaluation of this relationship yields a correlation ($\gamma = .57$) which is just significant at the 5% level. Thus differential rate of absorption of D-phenylalanine is of some importance in explaining the differential excretion of this substance.

We may next consider the possibility of the metabolism of ingested D-phenylalanine. There is considerable evidence(1,6) that D-phenylalanine may be utilized to some extent by man. Such utilization would require inversion of the D to the L form or oxidative deamination of D-phenylalanine to phenylpyruvic acid. These are not necessarily independent or mutually exclusive processes, since, in fact, inversion might require prior oxidation to phenylpyruvic acid.

In the fourth and fifth columns are given the fasting and the post-feeding plasma levels of L-phenylalanine. Any inversion of D to L should be indicated by an increase in the post-feeding plasma level of L-phenylalanine. Except for the second experiment on subject 2, there is no evidence for such conversion; in fact, the means of the 2 columns are equal. Therefore, if inversion of D to L is taking place in these experiments, the rate of inversion is so low that this process cannot be considered as contributing very much to the observed variation in D-phenylalanine excretion. However, in view of the evidence for

partial utilization of D-phenylalanine by man, it is most likely that inversion is occurring at a low rate in these experiments. Aside from experimental errors, there is an interesting point, peculiar to these experiments, which may explain our inability to detect inversion. This relates to the fact that D-phenylalanine through some inhibitive or competitive process increases the renal excretion of L-phenylalanine, thereby causing sufficient loss of plasma phenylalanine so that the effect of a slight amount of inversion (less than 5%) would be obscured. This phenomenon, together with further work, will be reported later.

In the next to last column is given the amount of phenylpyruvic acid excreted one hour after the feeding of D-phenylalanine. As can be seen, there is marked variation between individuals in this respect, with values ranging from less than 1 to over 15 mg of phenylpyruvic acid after the feeding of 0.5 g of D-phenylalanine. Considering this wide range of variation and the fair repeatability of experiments on the same individual, it would appear that this biochemical variation might be a profitable one for genetic studies. In this respect, it is interesting to point out that a similarly marked range of variation in the excretion of free phenols (presumably mainly p-hydroxyphenylpyruvic acid) following the feeding of D-tyrosine was observed by Albanese *et al.*(7). However, differences in the excretion of phenylpyruvic acid do not appear to account in any way for the observed variation in the excretion of D-phenylalanine. Small amounts of urinary phenylpyruvic acid would be associated with large amounts of urinary D-phenylalanine and vice versa, if variations in the excretion of D-phenylalanine were to be explained by differential excretion of phenylpyruvic acid. As can be seen, this is not the case, and furthermore, it is clear that the amounts of phenylpyruvic acid excreted relative to D-phenylalanine are small.

The final factor we should like to consider in the explanation of the variation of D-phenylalanine excretion is that of renal function. This could be an important factor if differential reabsorption of D-phenylalanine occurred in the tubules. To investigate this possibility, renal clearance values have been estimated and are given in the last column. Though these estimates of renal clearance were not obtained under rigorous physiological conditions, we feel that they do furnish a valid relative picture of the variation between individuals in the clearance of D-phenylalanine. As can be seen, there is a general correlation between the renal clearance values and the amount of D-phenylalanine excreted, the actual value of this correlation being .74, which is significant beyond the 5% level. Therefore, it would appear that differential tubular reabsorption of D-phenylalanine is an important determinant of the observed variation in the excretion of D-phenylalanine.

Discussion. From the data presented, it appears that differences in metabolism of D-phenylalanine are relatively unimportant variables in accounting for the observed variation in urinary excretion of D-phenylalanine. It seems, rather, that differences in absorption and renal excretion account for most of the observed variability in urinary excretion. In fact, considering that the latter two variables are independent (the correlation between absorption and renal excretion is not significantly different from zero), it appears that the combined variation in absorption and renal excretion can account for practically all of the observed variation in urinary excretion of D-phenylalanine under these experimental conditions.

However, in terms of genetic potentialities, it seems that excretion of phenylpyruvic acid after ingestion of D-phenylalanine is the most promising of the variables investigated. This is so because of the marked variation between individuals in excretion of phenylpyruvic acid and the repeatability of experiments on the same person.

Summary. This study represents an investigation into the physiological basis of the differential excretion of D-phenylalanine in man after its oral administration. Of the processes investigated: adsorption of the substrate into the blood, inversion to the L form, oxidative deamination to phenylpyruvic acid, and renal function, differences in absorption and renal function were important in accounting for the differential excretion of D-phenyl-

alanine.

We should like to acknowledge the assistance of Barbara Lenes Mohandes and Jayne Rosenthal of Barnard College.

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Received July 10, 1957. P.S.E.B.M., 1957, v96.

Pyridoxal Phosphate (B_6-al-PO_4) Levels of Circulating Leukocytes in Maternal and Cord Blood.* (23468)

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In the later stages of pregnancy there exists a disturbance of Vit. B_6 metabolism. Following a tryptophan load test large amounts of xanthurenic acid appear in the urine(1) and the excretion pattern of tryptophan metabolites on chromatographic analysis deviates from the normal(2). This is immediately corrected by administration of relatively small amounts of pyridoxine(1,2). Furthermore pregnant women retain administered Vit. B_6 to a larger extent than do normal controls(3). The present report deals with the estimation of the coenzymatically available active form of Vit. B_6, namely pyridoxal phosphate, in the only readily accessible prototype tissue, *i.e.* leukocytes, in the blood of non-pregnant controls, mothers at term and in the cord blood of their babies.

Material and methods. Blood samples were taken from non-pregnant healthy women, women during delivery and from the cord blood of their babies. The pregnant women were divided into 3 groups. One group received no vitamin supplementation containing Vit. B_6. A second group had taken, during the course of their pregnancy, vitamin preparations which contained some Vit. B_6 in an uncontrolled and irregular manner. A third group had received, for a period of at least 14 days prior to their confinement, 2 tablets of 5 mg each pyridoxine hydrochloride daily. 5-10 ml venous blood was collected into siliconed test tubes containing a small amount of disodium versenate. Pyridoxal phosphate was estimated in isolated leukocytes by a recently developed method(4) which is based on the coenzyme function of B_6-PO_4 in a tyrosine decarboxylase system from *Streptococcus faecalis*(5). The leukocytes were isolated within a few hours after the blood had been obtained(5a). To the packed leukocytes 0.25 ml of 0.5 *N* NaOH was added and the tubes immersed in a boiling water bath for 5 minutes. The alkaline hydrolisates were kept in the refrigerator at 4°C and worked up within 24 hours. All analyses were made in triplicate and the samples were assayed at random.

Results. The results are summarized in Table I. In the first experimental group consisting of 60 non-pregnant, healthy women of child-bearing age, the amount of pyridoxal phosphate varied from 0.11 to 0.79 mγ/million leukocytes, averaging 0.32 ± 0.02 mγ/million leukocytes. The second group consisted of 51 women at term who had received no Vit. B_6 supplementation during their pregnancy. The B_6-al-PO_4 levels varied between

* This work was aided by grants-in-aid and material from Merck and Co. and National Vit. Fn. We are indebted for valuable advice to Dr. G. E. Boxer, Merck Inst. for Therapeutic Research.