XXXIII. THE CONCENTRATION OF DIASTASE IN THE URINE THROUGHOUT THE DAY.

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THE investigation forming the subject of this paper was a continuation of an enquiry into the origin and method of excretion of urinary diastase. The concentration of this enzyme in the urine during the various parts of the day, and the effect of meals over an observation period of 24 hours are specially dealt with.

In a previous paper [Cohen, 1925] it was shown that whereas the concentration of diastase in the blood remained remarkably constant over various periods of observation, that in the urine showed marked variations throughout the same periods. Following the intake of food there appeared a definite, though at times quite small, increase in the amount of diastase in the urine, but, as in some cases almost equivalent changes in concentration took place during starvation experiments, some doubt was expressed as to whether the taking of food was really the cause of this increase in concentration of the enzyme in the urine. Arising out of these facts it was obvious that the examination of isolated samples of urine for clinical purposes would tend to yield very unreliable information. For further research it became desirable to know the conditions under which one could collect a specimen of urine which would yield the most reliable results as a basis for judging deviations from the normal. In the course of some more experiments on the effect of meals on the output of urinary diastase, it was found that the response to meals appeared greatest after the first meal in the day. This is in agreement with Stocks [1916], who from the results of seven cases concluded that the concentration of amylase in the urine is at a maximum just after breakfast. This is as one might expect it to be, for it appears reasonable to suppose that after a night's rest the digestive system would respond more readily to stimulation by food than at some time during the day after meals have been taken. In the same way it was thought that the nightly rate of excretion of diastase, in view of the absence of complications due to meals, might give fairly constant results from night to night, and so this point was investigated.

The output of diastase during the night was obtained from experiments carried out on the following lines. The subject was instructed to empty the

bladder on retiring for the night, to discard this specimen, and to note the time. On rising in the morning the bladder was again emptied, the urine collected, and the time again taken. The volume of urine passed was measured, the diastatic index of the specimen was estimated, and from the data so obtained the rate of excreting units of diastase per hour was calculated. It was soon noticed that the same individual showed great variations in the rate of output of enzyme on different nights. For instance, in one case the same person showed during one night an excretion rate of 2508 units diastase per hour, and 15 days later a rate of only 350 units per hour. To obtain further information a consecutive series of nightly urines was collected, the same conditions being observed each night according to the following procedure, and the diastatic rate was determined. The method used for the latter in these experiments was the one described by Wohlgemuth [1908], and modified by Dodds [1922].

The individual on whom the experiment was carried out adhered to the following routine throughout the period of observation. Dinner was taken each day at about 7 p.m., and no more food or drink taken till breakfast the following morning. At 11 p.m. the bladder was emptied and this specimen was discarded. The interval of 4 hours between the meal and the commencement of observation was allowed in order to overcome as far as possible any effect the meal would have upon the urinary diastase. At 7.30 a.m. the next morning the night's urine was collected, its volume was measured and the diastatic rate calculated. It was seen after a few days that the results obtained varied markedly from night to night. The nightly rates of diastase output not yielding constant results, it immediately suggested itself that the most likely specimen of urine to show a constant diastatic concentration from day to day, would possibly be the one collected just before the breakfast meal. The advantage in this case was that this specimen was farthest away from the last meal and, moreover, it was taken after a night's rest. From the 8th day of investigation, therefore, it was decided in addition to the nightly urine to collect and estimate each day the half-hourly specimen from 7.30 to 8 a.m. The results so obtained are represented graphically in Fig. 1 along with the nightly ones. The observations were continued for a period of more than 2 months. Inspection of the curves will show at a glance the marked variations in both series of figures, which, however, rise and fall together throughout the period of observation. It will also be seen that the rate first thing in the morning is higher than the nightly one. On the 50th day there occurred in the half-hourly specimen an unexpected enormous rise in diastatic concentration although experimental conditions were unaltered. Similar unaccountable increases in a single half-hourly specimen will be referred to later. During the last 17 days of the experiment this same subject collected 24-hourly specimens of urine. The daily urine from 8 a.m. to 11 p.m. was collected and, with the other specimens collected as before, it was possible to deduce the average rate of excretion of diastase into the urine throughout the whole day.

These figures, together with the total 24-hourly output, are given in the following table, which also serves to demonstrate the manner in which the results were calculated in all these experiments.

Excretion of urinary diastase over periods of 24 hours.

Day of experiment	Volume cc.		Units diastase per cc.	Units diastase per hour	Units diastase 24 hours
	Total	Per hour			
47	1082	45	33	1485	35,706
48	1222	51	25	1275	30,550
49	1528	64	25	1600	38,200
50	1527	64	25	1600	38,175
51	1433	59	25	1475	35,825
52	1341	56	22	1232	29,502
53	1402	58	25	1450	35,050
54	1398	54	25	1350	34,950
55	1389	58	29	1682	40,281
56	1488	62	25	1550	37,200
57	1197	50	29	1450	34,713
58	1308	55	25	1375	32,700
59	1492	62	25	1550	37,300
60	2356	98	20	1960	47,120
61	1228	51	25	1275	30,700
62	890	37	29	1073	25,810
63	1368	57	33	1881	45,144

Fig. 1. Excretion of diastase in the urine during the night and first thing in the morning.

----- Half-hour specimen.

———— Night specimen.

From the figures in this table in conjunction with the curves in Fig. 1, one is in a position to compare the rates of excretion of diastase into the urine during the whole cycle of 24 hours, first thing in the morning and during the night. The curves in Fig. 1 show that the half-hourly specimen has a higher rate than the average rate during the night, a finding which is contrary to what one would expect. On the other hand, the average rate throughout the

whole 24 hours is higher than the average nightly rate alone, it is clear therefore that the rate during the daytime alone must be still higher than the nightly one. That is to say, during the food-taking period diastase is being excreted into the urine at a greater rate than during the time when the digestive organs are at rest, but it cannot be inferred from this that it is the partaking of food that has produced this effect.

The last column in the table shows that the total output of diastase each day was between 25,000 and 47,000 units. Based on theoretical considerations, *i.e.* taking 1500 cc. as the average amount of urine passed by an adult in 24 hours, and 10 to 30 units, on which most authorities agree, as the average diastatic index, one obtains a quantity of enzyme passed in 24 hours varying from 15,000 to 45,000 units. Harrison and Lawrence [1923] give the normal range as lying between 8000 and 30,000 units. In examining 24 hourly urines of patients other than nephritic and pancreatic cases, who are confined to bed in the wards, figures as low as 6000 units have been found. Patients on restricted diet also give low figures. Now it will be seen here, as has also been pointed out in previous work, that the approximate readings given by the method employed are partly responsible for the irregular curves in the table. For clinical purposes this standard method of Wohlgemuth's is still of great value, for only very high or very low figures are considered, but for research purposes a method is required which will give readings at least to the nearest whole unit. Such a method was described in conjunction with Dodds [Cohen and Dodds, 1924, 1]. Using this method, an investigation was carried out with reference to the excretion of diastase into the urine over a continuous period of 24 hours. Half-hourly specimens were taken from feeding and starving individuals, so as to observe the effect of meals. Hitherto the experiments had been carried out over much shorter periods and never during the night.

Five healthy men working in the Biochemical Department were selected as subjects, three of whom took food at the usual times and two of whom acted as starvation controls. The latter also refrained from drinking throughout the period of the experiment. Sleep was, of necessity, an impossibility. Any results obtained from these experiments are not without somewhat serious objection. Thus it can be argued that the subjects were being examined under pathological conditions, since no sleep was obtained. It is suggested that conclusions based on half-hourly specimens of urine are of greater value than any observations on the whole of the night's urine obtained when the subject wakes in the morning.

The subjects dined at 7 p.m. and the experiment was commenced at 9 p.m. The subjects emptied their bladders at the commencement of the observation and this specimen was discarded. At half-hourly intervals throughout the following 24 hours specimens of urine were collected. The volume of each specimen was measured as soon as passed, and a few drops of toluene were added in order to preserve the specimen. Estimations of the diastatic values

were begun immediately during the hours of the experiment, and continued on the following days till the 240 urines had been estimated¹. The results were recorded graphically as rates of passing units of diastase per hour. Two of these curves, one from an individual who took food at the usual meal times, and the other from an individual who fasted, are represented for the purpose of comparison in Fig. 2.

Fig. 2. Excretion of diastase in the urine over a period of 24 hours.

----- Normal. Arrows indicate meals. ————— Starving.

From the previous observations [Cohen, 1925], on meal and starvation effects over short periods, it was noticed that, firstly, there was a definite rise in diastatic output within $\frac{1}{2}$ to 1 hour following a meal, and secondly, during the correspondingly short starvation controls the amplitudes of the curves were not diminished, which might have been the case had the variations in the figures been due solely to the intake of food. On inspecting Fig. 2 it will be seen that after each meal, which is represented by an arrow in the normal curve, there is a rise in diastase output. The starvation curve should supply information as to whether these rises are really due to meals, for it would be expected that the effect of not taking food would produce a much smoother curve. Three marked rises are seen culminating at the points *A*, *B* and *C*. These rises are quite as large in amplitude as the rises after meals in the normal curve. It is remarkable that these points correspond approximately in time to what appears to be an effect due to meals in the normal curve. It must therefore be concluded that the increase in diastase output after a meal is not really due to the intake of food, but is a coincident rise which would probably have occurred had no meal been taken.

¹ Several other constituents, such as urea, total nitrogen, etc., were determined on these urines and the findings were recorded elsewhere [Cohen and Dodds, 1924, 2].

It has previously been recorded that in an experiment one half-hourly specimen without apparent cause showed an enormous increase in diastatic concentration. Two further such observations appeared in these experiments. One specimen showed an increase in rate to about 4000 units, the one preceding it being about 500 units and the one following about 700 units, and another increased from about 900 to 3000 units. These extraordinary figures are however not subsequently maintained, and have only a slight effect on the average rate taken over several hours. Such facts point out the danger of working with isolated specimens.

SUMMARY.

The rate of appearance of diastase in the urine is greater during the day-time than at night. This higher rate during the period of meals is not due to the taking of food. Variations occur from day to day in the total daily and nightly output of diastase in healthy individuals. It is therefore clear that no significance can be placed on figures unless they differ markedly from the normal over a period of several hours.

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