THE INHIBITION OF ACID PHOSPHATASES BY FORMALDEHYDE AND ITS CLINICAL APPLICATION FOR THE DETERMINATION OF SERUM ACID PHOSPHATASES

BY

M. A. M. ABUL-FADL

AND

E. J. KING

From the Postgraduate Medical School of London

(RECEIVED FOR PUBLICATION, DECEMBER 16, 1947)

Introduction

The presence of considerable amounts of an active phosphatase with pH optimum about 5, normally present in human prostate, was first described by Kutscher and Wolbergs (1935). Many other tissues and fluids of the body contain acid phosphatases in variable amounts, but these are small in comparison with those found in the prostate and seminal fluid.

Normal plasma and serum contain small amounts (1 to 4 units) of acid phosphatase. It was first observed by Gutman and Gutman (1938) that the concentration of the enzyme increases in the sera of patients with metastatic carcinoma of the prostate, presumably due to neoplastic invasion of lymph or blood channels, with the escape of the enzyme into the circulatory fluids. In many instances the increases are so great as to be clearly diagnostic; but in some cases there are only slight increases such as are occasionally found in other diseases. In such cases, therefore, a method for measuring the prostatic fraction of acid phosphatase is greatly needed.

On account of the marked susceptibility of the prostatic phosphatase to inactivation by certain narcotics, including alcohols, which was first reported by Kutscher and Wörner (1936), Herbert (1944, 1945, 1946) was able to devise a method for the specific estimation of prostatic phosphatase in serum. The basis of Herbert's method is that incubation of serum or plasma with (two-fifths volume) absolute alcohol at room temperature for 30 minutes completely destroys the prostate enzyme fraction but hardly affects the normal plasma acid phosphatase.

During the course of a careful study of this method we have tried the effect of alcohol incubation on serum acid phosphatase in a great number of cases of normal and non-prostate diseased persons. We have found that, while in many cases the serum acid phosphatase is not significantly affected by this alcohol treatment, yet in a good number of cases a marked inhibition is observed, for example in certain types of liver disorders and other diseases. In other cases the alcohol treatment leads to an actual, and unexplained, increase in the total acid phosphatase (cf. Appendix).

This directed our attention to the possibility that this lability of the prostatic enzyme to the destructive action of alcohol may be shared by other tissue phosphatases as well. It has been found that while many tissue acid phosphatases, including those of the adrenals, intestines, liver, spleen, etc., are not affected by alcohol incubation, the red cells, bile, and kidney enzymes are, on the other hand, seriously inhibited.

The red blood corpuscles are second to the prostate in regard to their high content of acid phosphatase, and contain nearly one hundred times the amount present in plasma. King and others (1945) found the acid phosphatase of prostate and red cells to be similar in many respects, including easy destruction by alcohol.

Thus, for applying Herbert's technique, the sera should be strictly free from any trace of haemolysis. This introduces great difficulty as it has been found in practice that the majority of samples of blood taken in the wards for analysis show variable degrees of haemolysis. Even when blood is taken with great care, unless the plasma is

separated as soon as possible, there is always the possibility of escape of the enzyme from the red cells into the plasma, even without detectable haemolysis. This has been found especially in cases when the corpuscles are abnormally rich in acid phosphatase. Many samples sent to the laboratory showing apparent haemolysis have to be reported "unfit for acid phosphatase determination."

For the above reasons it is felt that there is a distinct need for a more reliable method, sufficiently simple to be adopted in routine work, giving results of definite diagnostic values in prostatic cases, and not influenced by the corpuscular or other body tissue enzymes other than those of the prostate.

It has been found, after trying a series of organic substances belonging to many different groups, that formaldehyde answers many of these requirements. Under our experimental conditions, formaldehyde destroys completely the red-cell enzyme while not affecting the prostate enzyme to any appreciable degree. Other tissue acid phosphatases are variably inhibited by formaldehyde, but none is found to be so sensitive to this reagent as the corpuscular enzyme.

The normal serum or plasma acid phosphatases are also seriously damaged by formaldehyde. Thus, by applying a very simple formaldehyde technique, we can always get the prostatic fraction of serum acid phosphatase free, or nearly free, from the other interfering acid phosphatases.

Methods

The King and Armstrong (1934) phenyl phosphate method, as modified by Gutman and Gutman (1938, 1941) for the estimation of serum acid phosphatase, has been followed in principle. Certain modifications are introduced to suit either Herbert's technique or the proposed formaldehyde procedure, both of which are described here in detail.

REAGENTS

Acid citrate buffer.—pH 4.9. Disodium citrate, 21.25 g., dissolved in 1 l. distilled water containing 100 ml. N/10 HCl. The solution should be preserved with 5 ml. chloroform and kept in the cold.

Substrate.—M/50 disodium phenyl phosphate*: 2.18 g. dissolved in 500 ml. distilled water. The solution should be brought quickly to the boil to destroy any organisms, cooled immediately, and preserved with 5 ml. chloroform and kept in the cold.

* Commercial specimens of disodium phenyl phosphate may contain traces of free phenol, which add appreciably to the blank colour in the phosphatase method. Any free phenol is easily got rid of by washing the solid disodium phenyl phosphate with ether, after which it should be dried in the air.

Folin-Ciocalteu phenol reagent (1927).—Diluted 1 in 3.

25 per cent sodium carbonate (anhydrous) (w/v).

—This solution should be kept in a warm place.

Standard-phenol-and-reagent.—(1 mg. phenol/100 ml.) 5 ml. of stock standard phenol (100 mg./100 ml.) is accurately measured into a 500-ml. volumetric flask; 100 ml. of dilute (1:3) Folin-Ciocalteu reagent is added, and water to the mark.

Formaldehyde reagent.—(2 per cent formaldehyde solution.) 5 ml. of neutral formaline solution (40 per cent) is pipetted into a 100-ml. measuring flask and diluted to the mark with distilled water.

Procedures

Fresh serum or plasma must be used, and examined with the visual spectroscope, if necessary, to be sure of complete absence of haemolysis whenever the alcohol procedure is to be adopted.

Total acid phosphatase.—1 ml. substrate and 1 ml. distilled water are pipetted into each of two centrifuge tubes together with 2 ml. of the buffer solution. The tubes are allowed to remain in a 37° water-bath for three minutes to allow the contents to attain the temperature of the bath. After this time 0.2 ml. of the serum is added to one of the tubes and the enzyme hydrolysis allowed to proceed for exactly one hour, at the end of which time 1.8 ml. of Folin and Ciocalteu's phenol reagent are added to each tube and 0.2 ml. of serum to the second tube. The two tubes are shaken and centrifuged, and 4 ml. of the supernatant pipetted into two test tubes. After the addition of 1 ml. of 25 per cent sodium carbonate solution, the tubes are replaced in the water-bath for fifteen minutes to allow the colours to develop. The colour intensity is compared with that of a standard solution of phenol (i.e., 4 ml. of standard-phenol-and-reagent and 1 ml. of sodium carbonate) which has been similarly treated. A photo-electric colorimeter is used for this purpose, setting its zero with water, or, better, a blank of 3 ml. water, 1 ml. phenol reagent, and 1 ml. sodium carbonate solution (without serum), and using a red filter. The difference between the incubated and non-incubated samples gives the phenol formed by hydrolysis. A unit of acid phosphatase is defined as the amount of enzyme which will liberate 1 mg. phenol per 100 ml. serum or plasma per hour from M/200 disodium phenyl phosphate substrate at a pH near 5 at 37° C. (If the colours are too deep for reading in the available colorimeter, the coloured solutions (test, control, blank, and standard) should be diluted to 10 ml. with water.)

Calculation :

$$\left(\frac{T}{S} \times 0.04 \times \frac{6}{4} \times \frac{100}{0.2 \text{ test}} \right) - \left(\frac{T}{S} \times 0.04 \times \frac{6}{4} \times \frac{100}{2 \text{ control}} \right) \\ = \left(\frac{T}{S} \times 30, \text{ for test} \right) - \left(\frac{T}{S} \times 30, \text{ for control} \right) = \text{units phosphatase/100 ml. ;}$$

where T=reading of test; S=reading of standard; 0.04=mg. phenol in the 4 ml. of standard-phenol-and-reagent; 6=the vol. of 1 ml. substrate+1 ml.

water+2 ml. buffer+0.2 ml. serum+1.8 ml. Folin-Ciocalteu; 4=the vol. of protein-free supernatant taken; and 0.2=the vol. of serum.

Alcohol inactivation.—To 1 ml. of serum or plasma, 0.4 ml. of absolute ethyl alcohol is added. After shaking, the tube is stoppered and the mixture allowed to stand for half an hour at room temperature. Water (0.6 ml.) is added, and a sample of 0.4 ml. of the opalescent mixture (corresponding to 0.2 ml. of serum or plasma) is added to the warm, previously mixed, 2 ml. buffer+1.0 ml. substrate and 0.8 ml. water to make a final volume of 4.2 ml. buffer-substrate-enzyme mixture. The procedure is then followed exactly as above for total acid phosphatase.

Formaldehyde technique.—The procedure is exactly as described above under "total acid phosphatase" except that, in this case, 1 ml. formaldehyde reagent is added instead of the 1 ml. distilled water to the mixture of 2 ml. buffer and 1 ml. substrate solutions.

All the following details are then the same.

Experimental

THE RELATIVE CONTENT OF "ACID" AND "ALKALINE" PHOSPHATASES IN SOME HUMAN TISSUES

Since we are mainly concerned with the serum acid phosphatases, we thought it necessary to examine the various body tissues which are believed to be responsible for the variation of this enzyme in plasma in health and disease.

Although a good deal of work described in the literature has been carried out on various animal tissues in this connexion, very little has actually been done on human material. Normal adult tissues, without any apparent disease, were obtained from autopsy within 24 hours of death. After being freed from fat and other adhering extraneous tissue, they were carefully

washed with normal saline and then with cold tap water until they were as free as possible from any blood cells. They were then dried by filter paper, weighed and ground with sand, and mixed intimately in a mortar with an equal weight of normal saline solution and 10 per cent (by volume) of a mixture of equal parts of toluene and ethyl acetate. After having been left to autolyse at room temperature for two or three days with occasional shaking, the mixtures were centrifuged and the supernatant liquids filtered clear through a Whatman No. 1 folded filter paper.

Tissue extracts obtained in this way are always clear and free from any colour of blood, and their enzyme activity corresponds to that present in the original wet tissue. They keep their activity unchanged for several weeks if kept in the ice chest. They should be diluted ten to twenty times, or even more in certain cases, with normal saline just before their activity is determined. In the case of the red cells, the freshly drawn blood was directly centrifuged, the plasma separated, and the red cells washed twice with normal saline by spinning and decantation. 1 ml. of washed red cells was lysed in 9 ml. water and the solution centrifuged. The clear supernatant was used directly for determining its enzyme activity. The prostate gland was decapsulated, freed from adhering tissue, cut into fine pieces, and ground with sand in a mortar with five times its weight of normal saline solution. A few drops of toluene were added but no ethyl acetate. The mixture was left at room temperature to autolyse for two or three days with occasional gentle rotating. (Vigorous shaking should be avoided as it may inactivate the enzyme.) The mixture was then filtered through filter paper. It was highly diluted (e.g.—1,000 to 5,000 times) before determining its enzyme activity.

TABLE I
ALKALINE AND ACID PHOSPHATASE OF HUMAN TISSUES

Tissue	No. of specimens examined	mg. phenol liberated/hr./1 g. wet weight tissue				Ratio alkaline/acid
		Alkaline phosphatase		Acid phosphatase		
		Range	Average	Range	Average	
Adrenal glands	6	25-38	30	2-5.5	3.0	10
Bile from gall-bladder	5	10-23	16	0.3-7.0	2.5	6.2
Intestinal tissue	5	49-72	53	2-3	2.7	20
Kidney	6	12-24	16	6.5-18	10	1.6
Liver	6	12-37	14	7-13	9.7	1.4
Pancreas	5	0-6	3	3-11	5.7	0.5
Prostate	10	0.1-1.2	0.5	2,000-10,000	4,000	0.00025
Red cells	20	0.6-2.5	1.5	8-25	15	0.1
Spleen	5	10-17	14	4.5-8.5	6.7	2.0
Thyroid	4	2-4	3	1.5-3	2.0	1.5

The phosphatase activity of these preparations was determined at two hydrogen ion concentrations; namely, pH 5 and pH 10, using the above-described acid citrate buffer for the first, and alkaline carbonate-bicarbonate buffer (Delory and King, 1945) for the latter, with disodium phenyl phosphate as substrate. For the sake of comparative study of the relative activity of the two enzymes at these two pH values, the buffer-substrate-enzyme mixtures were incubated for 60 minutes at 37° C. The phenol liberated at the end of that time was taken as an index of the enzyme activity at the corresponding pH.

Thus Table I gives the results expressed in mg. phenol liberated per 1 g. wet tissue per hour at the two given pH values. (No Mg was added.)

Table I does not include the bone phosphatases, which, in the normal adult, are not expected to be of much activity. The intestines are the richest human source of alkaline phosphatase, followed by the suprarenals. The human kidney seems to be very poor in alkaline phosphatase compared with other animals' kidneys, while the human liver appears to contain much more alkaline enzyme than that of other animals.

As for the acid phosphatases, the prostate is unquestionably the richest source in the human. The only other animal in which it occurs in large amounts is the monkey and then only in the caudal lobe of the prostate, as shown by Gutman and Gutman (1939). The second tissue that is rich in acid enzyme is the red cell, although the difference is very great. Red cell phosphatase varies enormously in different individuals, for no known reason, but generally speaking it has been found to diminish in activity with age.

The human kidney, liver, pancreas, and spleen contain appreciable amounts of acid phosphatase.

EFFECT OF ALCOHOL INCUBATION AND FORMALDEHYDE TREATMENT ON DIFFERENT TISSUE ACID PHOSPHATASES

The tissue extracts described above were subjected to both alcohol incubation and formaldehyde treatment in the same way as described for serum. Table II shows the result of several experiments carried out in this connexion on different tissue extracts.

From Table II the inhibitory action of alcohol on prostate, bile, kidney, and red cells is evident.

The failure to achieve 100 per cent inactivation of the prostate enzyme by alcohol incubation can be explained by the fact that we were working with enzyme solutions in normal saline. Herbert showed that certain conditions must be fulfilled in

TABLE II

EFFECT OF ALCOHOL AND OF FORMALDEHYDE ON ACID PHOSPHATASES

Tissue preparation	Acid phosphatase units/100 ml.*	Percentage inhibition	
		After alcohol	With formaldehyde
Adrenals ..	21-64	0	12-25
Bile ..	1.5-34	80-100	20-50
Intestines ..	35-71	0	40-50
Kidneys ..	37-153	17-62	24-50
Liver ..	35-92	0	30-60
Pancreas ..	30-60	0	15-40
Prostate ..	25-300	40-70	0
Red cells ..	9-120	40-55	100
Spleen ..	17-127	0	44-52
Thyroid ..	20-75	0	25-40

* These figures represent the enzyme activities of extracts of tissues at various dilutions prepared to give a convenient concentration of phosphatase for accurate measurement. They do not represent the comparative enzyme contents of the tissues themselves.

order to get complete inactivation of the prostate enzyme solution with alcohol incubation; for example, the presence of a sufficient bicarbonate concentration and other serum salts of weak acids and strong bases is essential.

The destruction of the bile acid phosphatase with alcohol is especially striking, since that of the liver is not affected. It might be possible that this inhibition is indirectly due to certain other bile constituents affected by the alcohol incubation. Until both the liver and the bile enzymes are prepared in a purer state, we cannot decide if there is a real difference between them.

Formaldehyde inhibits the different tissue enzymes to variable extents (20 to 60 per cent), but its 100 per cent inhibitory action on red cells is remarkable; likewise its complete lack of any effect on the prostatic phosphatase.

The difference between the red-cell enzyme on the one hand, and the liver and spleen enzymes on the other, which was first described by Davies (1934), is further shown here in regard to their behaviour towards alcohol and formaldehyde.

EFFECT OF ALCOHOL INCUBATION AND FORMALDEHYDE TREATMENT ON NORMAL PLASMA AND ON PROSTATE AND RED CELL SOLUTIONS IN PLASMA

The following illustrations of the effect of alcohol incubation and formaldehyde treatment on normal plasmas alone, and on those to which prostate extracts and red-cell solutions have been added, form the basis for the clinical application of these methods.

It has been shown by Herbert that in the case of alcohol incubation the behaviour of the prostate enzyme solution in normal saline differs from that in serum or plasma. It is more strongly inhibited when present in the latter state than when it is merely in saline solution. Thus the question arises as to whether the prostate and red-cell enzyme solutions in water will behave differently to those in serum or plasma with regard to the formaldehyde treatment. The answer is found in Table III, from which it is seen that in plasma or serum media the formaldehyde still exhibits its strong inhibitory

action on the red-cell enzyme while not affecting at all the prostate phosphatase.

Normal plasma phosphatases are sensitive to formaldehyde but resistant to alcohol. The alcohol incubation seems to destroy totally the prostate enzyme in serum or plasma medium, while the red-cell enzyme is only partly destroyed.

The formaldehyde, however, provides a quantitative means for estimating the prostate enzyme in the presence of the red cell. This is clearly shown in Table IV, in which two solutions, one of prostate extract and the other of red cells, both of known enzyme activity, are mixed together in different proportions and subjected to formaldehyde treatment. The residual values after formaldehyde treatment always agree closely with those corresponding to the amount of prostate enzyme added. Table IV shows that a quantitative determination

TABLE III

EFFECT OF ALCOHOL AND FORMALDEHYDE ON PLASMA PHOSPHATASE ENRICHED BY THE ADDITION OF PROSTATE AND RED-CELL PHOSPHATASES

	Acid phosphatase units/100 ml.	After alcohol incubation	After formaldehyde treatment
1. Normal plasma	4.5	4.0	1.1
" " + prostate ext. ..	48.6	6.3	45.0
" " + prostate ext. ..	73.5	3.7	69.7
" " + red cells soln. ..	45.7	17.3	1.5
2. Normal plasma	6.0	5.2	1.5
" " + prostate ext. ..	100.5	6.7	95.2
" " + red cells soln. ..	37.5	24.0	3.7
" " + red cells soln. ..	66.0	15.0	3.2
3. Normal serum	3.0	4.0	1.5
" " + prostate ext. ..	48.0	3.0	47.2
" " + red cells soln. ..	24.0	13.2	1.5
4. Plasma	3.5	4.6	1.5
" " + prostate ext. ..	49.5	4.5	47.5
" " + red cells	31.5	10.5	1.5

TABLE IV

AGREEMENT BETWEEN THE CALCULATED AND DETERMINED ACID PHOSPHATASE CONTENTS OF PROSTATE-RED-CELL MIXTURES

	Units/100 ml. solution		Calculated units of prostate enzyme in mixture
	Without formaldehyde	With formaldehyde	
Solution (A) prostate ext.	93	93	
Solution (B) blood cells	30	0	
Mixture of (A) + (B)			
1(A) + 1(B)	64.5	48.0	46.5
1(A) + 2(B)	51.5	31.5	31.0
2(A) + 1(B)	73.0	62.0	62.0

TABLE V

INHIBITING EFFECT OF VARIOUS CONCENTRATIONS OF FORMALDEHYDE ON TISSUE ACID PHOSPHATASES

	Units/100 ml. before treatment	Units/100 ml. after treatment with formaldehyde			
		1%	0.5%	0.25%	0.1%
Normal serum	4.3	1.5	1.5	3.3	3.7
Prostate extract	150.0	140.0	148.5	150.0	150.0
Normal serum + prostate ..	45.5	39.8	42.5	43.5	44.0
Red cells in water	40.0	0	0	0	0
" " " "	140.0	0	0	12.0	15.0
Normal serum + red cells ..	24.0	1.5	1.5	3.0	3.5
Adrenals	21.0	16.5	—	—	21.0
Kidney extract	38.6	20.5	21.5	22.6	24.6
Liver	35.5	18.7	21.4	22.0	23.6
Pancreas	13.5	11.2	11.2	—	11.2
Spleen	56.2	24.1	26.8	27.5	—

of prostate and red-cell enzymes, when present in a mixture, can be easily effected.

EFFECT OF VARIOUS CONCENTRATIONS OF FORMALDEHYDE ON DIFFERENT ACID PHOSPHATASES

The concentration (final) of formaldehyde, so far used in all the previous experiments, and which is suggested for use in routine work, is 0.5 per cent in the enzyme-substrate-buffer mixture.

This concentration has been found experimentally to be the maximum that can be used without affecting the prostate enzyme, while producing its maximum inhibition of the serum acid phosphatases. Table V shows the effect of various concentrations of formaldehyde. The extreme sensitivity of the red-cell enzyme to formaldehyde is apparent. As little as 0.1 per cent of this reagent completely destroys small amounts of the enzyme, while inhibiting nearly 90 per cent red-cell preparations of high phosphatase activity (for example, 140 units/100 ml.). On the other hand solutions of formaldehyde stronger than 0.5 per cent affect the prostate enzyme. The plasma and serum acid phosphatases seem not to be inhibited beyond certain limits even with increase of formaldehyde concentration. The 0.5 per cent concentration of formaldehyde seems, therefore, to be just suitable for the purpose.

CLINICAL APPLICATION

The alcohol and formaldehyde techniques have been applied to a variety of plasmas and sera from different types of disease, covering 67 prostate cases, 24 cases of other tissue cancers, 15 cases of bone disease, 38 cases of liver disease, 16 cases of kidney disease, and 56 cases of other miscellaneous diseases.

The majority of these samples were subjected to both formaldehyde and alcohol incubation procedures, except in some instances where there was either shortage of material or where the samples showed slight signs of haemolysis. The majority of samples were collected from the Hammersmith Hospital, others from other London hospitals, and the sera and plasmas, which were separated as soon as the blood was received, were kept in the ice chest until the tests could be carried out (usually without delay).

Results and Discussion

A synopsis of the results of the clinical application of both alcohol incubation and the formaldehyde method to pathological sera and plasma is given in Table VI and the results appear in full in the appendix.

For convenience the "total acid phosphatase" figures are classified into two main groups: "normal values" (those up to 5 units); and "abnormal values" (more than 5 units per 100 ml.).

TABLE VI

SUMMARY OF ACID PHOSPHATASE RESULTS

(Total acid phosphatase units in roman type, formaldehyde-stable in italics)

Diagnosis	Number of cases with acid phosphatase in the range												
	0 to 1.5	1.6 to 3	3.1 to 5	5.1 to 7	7.1 to 10	10.1 to 15	15.2 to 20	21 to 30	31 to 40	41 to 50	51 to 70	71 to 100	>100
Carcinoma of prostate with bone metastases (22)*	—	2	4	1	1	2	4	2	—	2	—	—	4
Carcinoma of prostate; no bone metastases demonstrable (22)	4	2	2	—	3	2	2	1	1	1	—	—	4
Senile hypertrophy, and retention with enlarged prostate (23)	—	4	6	4	4	3	—	—	—	—	—	—	1
Cancer of organs other than prostate (24)	5	9	5	1	—	1	—	—	—	—	—	—	1
Paget's disease of bone (15)	—	9	4	3	2	—	3	2	—	—	—	—	—
Obstructive jaundice (18)	8	8	1	2	2	—	1	1	—	—	—	—	—
Other liver disease (hepatitis, cirrhosis, etc.) (20)	—	3	8	5	4	4	—	—	—	—	—	—	—
Nephritis (16)	9	12	3	—	—	—	—	—	—	—	—	—	—
Miscellaneous (56)	11	4	5	4	1	1	—	—	—	—	—	—	—
	1	2	2	7	4	1	1	—	—	—	—	—	—
	2	7	6	2	1	—	—	—	—	—	—	—	—
	—	7	5	1	4	1	1	1	—	—	—	—	—
	7	6	5	2	—	—	—	—	—	—	—	—	—
	2	7	1	3	1	2	—	—	—	—	—	—	—
	7	4	3	2	—	—	—	—	—	—	—	—	—
	5	18	16	6	3	3	3	2	—	—	—	—	—
	29	20	3	4	—	—	—	—	—	—	—	—	—

* Number of cases.

PROSTATE CASES

The prostate cases examined, although not great in number, furnish a useful conclusion as to the successful clinical application of the formaldehyde technique. These cases are as follows:

Cases of carcinoma of prostate with bone metastases.—These are 22 in number; 16 of them gave abnormal values (>5) for the total acid phosphatase (73 per cent). Compared with the latest published results, Herbert (1946) found that about 83 per cent of 35 cases of prostatic cancer showing bone secondaries gave acid phosphatase values above 5, while Woodard and Dean (1947) found 72 per cent of 71 cases examined to give abnormal values.

Considering the formaldehyde-stable fraction in cases examined in the present work, it is noted that 16 cases out of the 22 (73 per cent) gave values above 3 units, while 14 of the latter gave values above 5 units. This means that if we adopt the figure 3 as the maximum permissible normal value for the formaldehyde-stable serum acid phosphatase (see below), we still get the same percentage of abnormal values.

The formaldehyde-stable fraction in most abnormal cases coincides fairly well with the alcohol-labile fraction, that is, with the difference between the total acid phosphatase and the alcohol-stable fraction (except in a few cases where the alcohol-stable fractions were unexpectedly high). If the two figures (after both alcohol incubation and formaldehyde treatment) be added together, values very near to the original total acid phosphatase are obtained (see Appendix).

The formaldehyde procedure provides a more reliable means of following the progress of cases under oestrogenic treatment than does the total acid phosphatase. High "total" values have shown a pronounced tendency to fluctuation, whereas the formaldehyde-stable figures steadily decreased during the course of favourable responses to treatment.

Cases of carcinoma of prostate without bone metastases (as shown by histological and radiological examination).—These are 22 in number. In 12 of them (55 per cent) the total acid phosphatase figures are above normal (i.e., >5); while with formaldehyde only 7 (32 per cent) gave values above 3 units and only 3 (14 per cent) gave values above 5 units. In one case only was the acid phosphatase very high (>100): repeated radiological examination failed to reveal any evidence of bone secondaries, until, in the latest film, an apparent abnormality was noted in one of the lumbar vertebrae.

Other prostate cases.—These comprise 23 cases of adenoma, acute retention with enlarged prostate, and senile hypertrophy. Most of these cases were examined radiographically and no evidence of bone lesion was found. The prostate growths were found histologically to be benign.

Ten cases (45 per cent) showed abnormal total acid phosphatase values while 7 (32 per cent) gave formaldehyde-stable figures above 3 units, and 6 (27 per cent) gave values above 5 units.

NON-PROSTATIC CASES

Cancer of various organs.—In this group figures are given for 24 cases of cancer of different organs other than the prostate. In some of these there were secondaries in the bones, and the alkaline phosphatase in one case is very high (85 units). In many of the cases there is a tendency to an increased total acid phosphatase value, not necessarily, however, paralleled by an increase in the alkaline phosphatase: for example, the highest total acid phosphatase value (15 units obtained in a case of cancer of bone) was accompanied by an alkaline phosphatase of only 23 units; while the case of carcinoma of the bronchus, invading several organs including bone, with the very high alkaline phosphatase (85 units), had only 3.4 total acid phosphatase units.

Of the 24 cases examined, 13 gave abnormal total acid phosphatase values (that is, above 5 units; about 54 per cent). With the formaldehyde technique none gave values above 5 units, and only 3 cases (about 12 per cent) gave values above 3, the highest of them being only 4 units.

Paget's disease of bone.—Fifteen cases of bone disease were studied. All of them were Paget's. Five are moderately high and one high. In Paget's disease there is no parallelism between the total acid phosphatase and the increased alkaline phosphatase values.

It is known that the phosphatase of bone is very sensitive to an acid pH, and one would not, therefore, expect any activity on the acid side of neutrality from bone phosphatase preparations, however high their activity might be on the alkaline side. Consequently, any increase in acid phosphatase in bone disease should be considered of non-osseous origin. In secondary carcinoma of bone, with the primary growth in organs other than the prostate, there might be a tendency, however, to high total acid phosphatase values. This was found to be the case in two sera examined. But formaldehyde treatment reduced all values obtained in bone disease to a range from 0 to about 3 (except in one Paget's, where it was 5.5).

Some authors are of the opinion that an extremely high serum alkaline phosphatase derived from bone may show its activity slightly at the acid reaction. But formaldehyde will exclude this completely, as the alkaline bone enzyme is very sensitive to this reagent. In the one Paget's case with an alkaline phosphatase of 140, and a formaldehyde resistant phosphatase of 5.5 (out of a total acid phosphatase of 7.5), the 5.5 value cannot be taken as a mere reflection of the very high alkaline phosphatase of bone origin. Such elevated phosphatases must be of non-osseous origin.

The possibility of the local production of acid phosphatases in tumours and abnormal active growths should not be neglected. Gutman and others (1936) observed that, in addition to alkaline phosphatase, acid phosphatase was markedly increased at the site of osteoplastic metastases secondary to carcinoma of the prostate gland. This was confirmed by Barringer and Woodard (1938). This acid phosphatase may be different from that of the prostate since it seems to be formaldehyde labile in most cases, as can be judged from the high total acid phosphatase levels, compared with the comparatively small formaldehyde-stable values, in cases of carcinoma of the prostate with bone metastases during treatment with oestrogens.

Liver disease.—The most striking incidence of high values of non-prostatic acid phosphatases occurs in liver diseases. 21 cases out of the 38 studied gave raised total values above 5 units. As has been previously pointed out, the bile acid phosphatase, which might account for some of the high values found in jaundice and other liver disease, is completely destroyed by the alcohol incubation and partially by formaldehyde. On the other hand the liver acid phosphatase is not attacked by alcohol and only partly by formaldehyde. The results of alcohol incubation and formaldehyde treatment, shown in the table, suggest that the abnormal amount of acid phosphatase present is derived both from the liver and from the bile, since it is only partly destroyed by both alcohol and formaldehyde in most cases.

Although formaldehyde treatment does not furnish a complete differentiation in cases of liver disease which show a tendency towards elevated formaldehyde-stable values (reaching 7.5 in 1 case), nevertheless it is still the most useful indicator we have (although some other means of sharper differentiation may eventually be found).

Miscellaneous diseases.—The result of applying the inactivation methods to the serum acid phosphatases in a variety of other diseases indicates that there is a tendency for an increased acid

phosphatase in some kidney cases. The alcohol incubation method affected quite a number of them. In a total of 53 cases (all free from haemolysis) subjected to the alcohol incubation method, 13 showed reduction of more than 1 unit.

As regards the formaldehyde treatment, it is noted that out of the total of 72 cases examined by this technique, 60 (i.e., 84 per cent) of them gave formaldehyde-resistant values between 0 and 3 units, while 6 (8 per cent) gave figures of 3 to 5, and 6 above 5 units.

It is striking that in some cases, for example, of anaemia, there is a small total acid phosphatase which is totally resistant to formaldehyde. This is not necessarily to be taken as of prostatic origin, for it occurs in both male and female cases. There may be present in some tissues not yet shown an acid phosphatase which is similar to the prostate enzyme in its stability towards formaldehyde, but which is not destroyed by alcohol.

Conclusion

The value of 5 units of total acid phosphatase, arbitrarily agreed upon by several workers as the maximum (normal figure) for non-prostate cases, has been exceeded in a good number of non-prostate cases. It is desirable to have a method giving a more restricted range in non-prostatic cases while not affecting the prostatic acid phosphatase. This has been satisfactorily found in the proposed formaldehyde technique.

Although the number of cases examined (216 in all) is not great, yet we believe we have sufficient data to support the following suggested limits for the formaldehyde-stable acid phosphatase:

Normal values: from 0–3 units per 100 ml. serum or plasma.

“Suspicious”: from 3.1–5 units per 100 ml. serum or plasma.

Prostatic: above 5 units per 100 ml. serum or plasma.

In most cases the simple formaldehyde treatment will serve to distinguish between the high acid phosphatase of prostatic origin and those accompanying other conditions. In a few cases this will not suffice, and Miss Herbert's technique of alcohol treatment should then be employed. But experience seems to show that certain liver cases (and possibly some cases of bone disease) are the only instances where confusion with prostatic disease is likely to arise, and here an alkaline phosphatase, together with other characteristic chemical and clinical tests, should resolve any ambiguity.

In addition to being useful for the study of special and difficult cases, formaldehyde and alcohol have a definite utility, along with other inhibitors and activators, in attempts to differentiate the several acid phosphatases of the different tissues of the body. We have evidence, for instance, of at least 2 acid phosphatases in most tissues and of there only being one in the prostate.

Summary

1. A study of the relative content of the acid and alkaline phosphatases of some human tissues has been made.

2. The effect of alcohol incubation was tried on these tissue acid phosphatases, and it has been shown that while those of the adrenals, intestine, liver, pancreas, spleen, and thyroid are not affected, those of the bile, kidney, red cells, and prostate, on the other hand, are seriously inhibited.

3. The effect of formaldehyde on the acid phosphatases of prostate and other tissues, including the red cells, was studied under certain conditions. Its complete destructive action on the red-cell enzyme, and its lack of any effect on the prostate enzyme under the same conditions, are reported. The other tissue acid phosphatases are variably inhibited, but none is similar to the red-cell enzyme in being completely inactivated.

4. The introduction of a formaldehyde technique in routine determinations of the serum or plasma acid phosphatase is suggested, and a method described.

5. The alcohol incubation method and the formaldehyde technique were applied to 216 pathological sera and plasmas, including prostate cases

and other cases of a wide variety of diseases. The results are discussed, and limits for the formaldehyde-stable fraction of serum acid phosphatase, for what should probably be considered non-prostate, are suggested.

6. A high acid phosphatase after formaldehyde treatment (above 5 units) strongly suggests a prostatic origin (values >3 are "suspicious"), and in most cases it gives useful information for distinguishing between raised values due to the presence of prostatic phosphatase, and those of different origin. An alcohol incubation test gives further confirmatory evidence in doubtful cases.

7. The formaldehyde technique permits the use of haemolysed sera or plasmas which are otherwise unfit for acid phosphatase determination.

We are indebted to Dr. E. P. Sharpey-Schafer, Dr. Jean Watkinson, Dr. G. A. Harrison, Dr. N. F. MacLagan, Dr. G. Discombe, Dr. F. T. Prunty, Prof. J. R. Marrack, and Dr. J. Goodie for samples provided.

REFERENCES

- Barringer, B. S., and Woodard, H. Q. (1938). *Trans. Amer. Ass. gen.-urin. Surg.*, **31**, 363.
 Davies, D. R. (1934). *Biochem. J.*, **28**, 529.
 Delory, G. E., and King, E. J. (1945). *Biochem. J.*, **39**, 245.
 Folin, O., and Ciocalteu, V. (1927). *J. biol. Chem.*, **73**, 627.
 Gutman, A. B., and Gutman, E. B. (1938). *J. clin. Invest.*, **17**, 473.
 Gutman, A. B., and Gutman, E. B. (1939). *Proc. Soc. exp. Biol.*, **N.Y.**, **41**, 277.
 Gutman, E. B., and Gutman, A. B. (1941). *Proc. Soc. exp. Biol.*, **N.Y.**, **47**, 513.
 Gutman, E. B., Sproul, E. E., and Gutman, A. B. (1936). *Amer. J. Cancer*, **28**, 485.
 Herbert, F. K. (1944). *Biochem. J.*, **38**, xxiii.
 Herbert, F. K. (1945). *Biochem. J.*, **39**, iv.
 Herbert, F. K. (1946). *Quart. J. Med.*, **15**, 221.
 King, E. J., and Armstrong, A. R. (1934). *Canad. med. Ass. J.*, **31**, 376.
 King, E. J., Wood, E. J., and Delory, G. E. (1945). *Biochem. J.*, **39**, xxiv.
 Kutscher, W., and Wolberg, H. (1935). *Hoppe-Seyl. Z.*, **236**, 237.
 Kutscher, W., and Wörner, A. (1936). *Hoppe-Seyl. Z.*, **239**, 109.
 Woodard, H. Q., and Dean, A. L. (1947). *J. Urol.*, **57**, 158.

APPENDIX

PLASMA ACID PHOSPHATASE (TOTAL, ALCOHOL-STABLE, AND FORMALDEHYDE-STABLE) IN PATHOLOGICAL SERA

Case	Acid phosphatase (units/100 ml.)			Alkaline phosphatase (K.A. units/ 100 ml.)	Notes
	Total	Alcohol- stable	Formal- dehyde stable		
PROSTATE CASES (67)					
Carcinoma of prostate with bone metastases (22)					
14.1.47	—	—	—	—	Admitted to hospital. Very ill
20.1.47	119	19	119	127	10 mg. oestradiol (i.m.) + 20 mg. stilb- oestrol (0.) daily for 8 days
29.1.47	55	5.8	49	120	Little improvement
4.2.47	39	—	33	128	40 mg. stilboestrol daily for 10 weeks
26.2.47	17	—	11	86	Improvement
3.3.47	17	—	12	76	
10.3.47	17	—	11	142	
14.3.47	23	—	6.0	180	
17.3.47	13	—	0	150	
19.3.47	9.0	—	1.5	118	Intermittent oedema
23.3.47	9.0	—	4.5	105	
26.3.47	7.5	—	3.0	58	Condition eased
2.4.47	8.2	—	3.7	65	
9.4.47	3.0	—	1.8	44	

Case	Acid phosphatase (units/100 ml.)			Alkaline phosphatase (K.A. units/ 100 ml.)	Notes
	Total	Alcohol- stable	Formal- dehyde- stable		
PROSTATE CASES (67) (continued)					
1 (contd.). Carcinoma of prostate with bone metastases (22) (contd.).					
17.4.47	22	—	6.6	48	Liver enlarged 200 mg. stilboestrol orally daily for 30 weeks
24.4.47	14	—	7.3	43	
1.5.47	17	22	5.1	40	Discharged on 200 mg. stilboestrol daily. Greatly im- proved, able to walk Liver not palpable Condition still good
8.5.47	21	23	5.1	43	
12.5.47	8.2	8.0	2.1	39	
15.5.47	9.2	10	3.7	38	
22.5.47	12	—	3.5	36	
29.5.47	13	—	5.0	36	
5.6.47	11	—	3.0	45	
12.7.47	4.7	3.7	2.2	33	
23.8.47	—	—	—	—	
29.9.47	9.5	11	0	9.6	
29.10.47	6.0	6.0	3.0	13	

Case	Acid phosphatase (units/100 ml.)			Alkaline phosphatase (K.A. units/ 100 ml.)	Notes
	Total	Alcohol- stable	Formal- dehyde- stable		

PROSTATE CASES (67) (continued)
Carcinoma of prostate with bone metastases (22) (continued)

2	145	2.7	145	—	
3	117	3.7	113	—	
4	113	0.5	112	—	
5	50	5.2	47	42	
	21	—	17	81	
	2.5	—	2	18	
6	45	—	38	—	Stilboestrol treatment
	10	4.5	4.2	24	
7	30	1.3	28	—	
8	21	0.8	20	—	
9	20	3.4	18	—	
10	19	—	14	12	
11	16	7.3	8.7	32	
12	11	3.7	8.2	18	
	18	—	9	11	
13	18	1.8	15	—	
14	11	—	4.5	—	
15	9	0.5	9	—	
16	6.8	5.4	4.8	37	
17	4.6	4.6	1.2	—	
18	4.5	0.7	2.3	—	
	1.2	—	0.6	5.2	
19	3.8	3.8	1.5	—	
20	3.7	3.4	2.0	14	
21	3	2.1	0.5	—	
22	3	1.9	0.8	—	

Carcinoma of prostate; no bone metastases demonstrated (22)

1	146	—	145	—	
2	14	3.0	12	15	
3	12	8.2	3.1	—	
	4.5	—	1.5	7.5	
4	11	8.8	2.5	—	
5	2.6	3.5	1.9	9	
6	9.5	—	3.7	14	
7	9	12	2.3	20	
	4.3	4.3	1.4	14	
8	7.9	—	6.8	—	Stilboestrol treatment
	3.9	—	1.5	—	
	2.5	—	1.4	—	
9	7.2	—	3.2	—	
10	6.8	5.4	4.8	16	
11	6.7	17	1.1	13	
12	6	0.7	0	—	Haemolysed
13	5.3	0.6	4.7	—	
14	4.7	—	1.3	—	
15	4.3	—	2.8	—	
16	4.2	3.3	3.0	—	
17	4.2	—	2.2	—	
18	4.0	4.5	1.8	—	
19	3.5	—	2.1	27	
20	3.0	2.0	3.0	—	
21	2.9	2.9	1.4	13	
	1.2	—	1.2	10	
22	2.9	—	0	—	

Other prostate cases (23)

1	30	—	23	9	Senile hyperplasia
2	23	—	7.5	—	Retention
3	20	—	7.5	—	Adenoma
4	19	7.5	17	—	"
5	17	—	4.5	23	Retention
6	10	6.2	6.9	—	"
7	8.5	6.5	5.8	10	"
	6.0	—	4.0	—	"
8	6.6	4.6	2.7	—	"
9	6.0	—	3.0	—	"
10	5.8	5.8	1.3	—	"
11	4.1	—	0	—	Adenoma
12	3.7	3.3	2.7	7.5	Senile hypertrophy
13	3.4	1.3	2.6	—	Retention
14	3.3	—	0	—	"
15	2.8	—	0.7	7.0	Adenoma
16	2.7	0.7	2.0	23	Senile hypertrophy
17	2.7	3.0	1.8	—	"
18	2.7	1.3	0.4	—	Adenoma
19	2.4	—	0	—	"
20	1.9	1.9	0.7	—	Retention
21	1.8	2.0	1.8	—	"
22	1.8	1.5	0.7	—	"
23	1.7	0.4	1.7	—	Obstruction

Case	Acid phosphatase (units/100 ml.)			Alkaline phosphatase (K.A. units/ 100 ml.)	Notes
	Total	Alcohol- stable	Formal- dehyde- stable		

CANCER OF VARIOUS ORGANS (24)

1	13	—	1.6	15	Cancer of bladder
2	15	14	3.0	23	" " bone
3	9.5	—	4.0	25	" " "
4	3.6	3.0	2.7	47	" " breast with metastases
5	9.0	10	2.4	50	" " breast
6	2.2	3.6	2.2	—	" " bronchi
7	3.4	3.7	0	85	" " "
8	6.2	3.0	2.7	8.5	" " "
9	2.7	2.1	1.3	5.2	" " colon
10	2.3	1.8	0.4	9.0	" " "
11	13	—	1.6	15	" " lung
12	11	—	3.0	—	" " "
13	8.3	—	1.6	17	" " "
14	7.5	7.5	1.9	—	" " "
15	6.0	—	2.3	45	" " "
16	5.1	—	3.3	—	" " "
17	4.8	3.5	0.6	11	" " "
18	3.7	—	1.5	—	" " "
19	5.4	3.6	1.2	30	" " liver
20	4.1	4.0	1.8	33	" " "
21	5.2	4.5	3.7	—	" " pancreas
22	4.5	3.0	1.5	—	" " oesophagus
23	6.4	9.3	1.1	—	" " rectum
24	3.6	3.0	0	7.8	" " "

PAGET'S DISEASE OF BONE (15)

1	12	7.6	3.3	20	
2	7.5	8.0	5.5	140	
3	6.7	6.0	0.4	15	
4	6.7	5.5	3.4	15	
5	6.3	5.4	2.9	75	
6	5.2	5.2	1.1	81	
7	4.5	3.0	1.5	76	
8	3.4	3.0	0.4	9.1	
9	3.1	2.9	0.8	30	
10	4.1	—	0.5	62	
11	4.0	—	1.3	—	
12	2.9	2.2	1.4	20	
13	2.7	—	0	—	
14	2.3	2.5	0	25	
15	2.3	—	0	—	

LIVER DISEASE (38)

1	9.6	—	1.2	17	Cholecystitis
2	2.5	2.0	0.2	—	" "
3	3.7	0.8	1.5	14	Cirrhosis
4	3.0	3.0	2.7	13	" "
5	2.2	0.9	0.4	22	" "
6	2.4	3.6	1.8	7.8	" "
7	18	15	3.7	—	Haematemesis, jaundice
8	7.8	6.8	3.8	150	" "
9	5.6	3.0	3.4	12	Hepatitis
10	2.5	2.7	2.5	—	" "
11	4.5	2.5	4.0	—	" "
12	4.8	4.4	3.0	28	" "
13	4.5	6.7	2.3	20	" "
14	12	11	1.4	21	" "
15	3.0	6.0	1.1	21	" "
16	22	15	5.6	19	Hepatomegaly
17	13	15	4.8	82	Obstructive jaundice
18	8.8	6.2	5.6	63	" "
19	8.2	4.5	3.0	32	" "
20	7.5	3.8	3.8	21	" "
21	7.2	6.5	5.7	136	" "
22	6.3	6.3	4.9	37	" "
23	6.0	5.3	4.5	17	" "
24	6.0	3.6	1.8	40	" "
25	5.4	3.6	3.0	23	" "
26	5.2	3.8	4.5	31	" "
27	3.8	3.8	2.5	14	" "
28	3.3	2.3	1.8	21	" "
29	2.7	1.8	1.8	80	" "
30	2.6	1.2	0.7	20	" "
31	19	14	7.5	44	" "
32	6.3	3.8	1.8	90	" "
33	6.0	3.5	4.5	40	" "
34	2.7	1.8	1.8	80	" "
35	2.6	1.3	1.9	40	Ulcerative colitis
36	8.0	5.3	6.0	114	Xanthomatosis
37	7.6	5.6	4.9	105	" "
38	3.2	3.2	3.0	122	" "

Case	Acid phosphatase (units/100 ml.)			Alkaline phos- phatase (K.A. units/ 100 ml.)	Notes
	Total	Alcohol- stable	Formal- dehyde- stable		

MISCELLANEOUS DISEASES

1	6.0	—	3.0	—	Anaemia
2	1.5	1.5	1.5	—	"
3	6.0	4.5	3.0	—	Anaemia haemolytic
4	3.8	—	1.5	17	" "
5	3.6	7.8	2.4	6	" " and oedema
6	16	—	5.2	—	" " pernicious
7	1.2	1.2	1.2	19	" " splenic
8	1.8	1.8	1.8	—	" " "
9	5.0	3.8	1.4	—	Arthritis
10	1.8	2.6	0.3	—	" (rheumatic)
11	1.8	1.2	0.7	—	Bronchopneumonia
12	1.6	—	0	—	" "
13	24	19	1.8	17	Cystitis
14	8.7	—	1.5	—	Diabetes
15	6.2	—	1.8	—	" and uraemia
16	2.3	2.3	1.4	11	Dysurea
17	2.0	0.5	0.5	—	Epididymitis
18	3.5	2.8	2.8	—	Fracture
19	3.8	—	2.3	—	Gout
20	4.8	4.2	2.0	5.5	Heart failure
21	3.7	3.7	2.2	—	" "
22	8.7	7.5	1.5	—	" " and hyper-
23	4.2	—	0	10	" " tension
24	2.2	2.0	0.7	—	Haemorrhage
25	2.5	—	1.6	—	" " accidental
26	12	—	6.0	—	" " "
27	2.2	1.5	1.8	—	Haematemesis
28	2.3	1.5	1.3	—	" "
29	17	19	4.5	—	Haematuria
30	7.5	6.8	3.0	—	Hodgkin's disease
31	4.7	3.3	2.7	—	Hypertension
32	2.4	1.8	0	34	Hypertensive heart
33	5.9	8.5	3.1	12	" disease
34	2.2	—	0.7	20	Hydronephrosis
35	7.0	7.0	3.0	—	

Case	Acid phosphatase (units/100 ml.)			Alkaline phos- phatase (K.A. units/ 100 ml.)	Notes
	Total	Alcohol- stable	Formal- dehyde- stable		

MISCELLANEOUS DISEASES (continued)

36	4.8	4.2	3.0	17	Melaena
37	1.8	1.3	0	6.6	"
38	25	17	0	14	Myeloid leukaemia
39	4.5	4.5	2.2	—	Myxoedema
40	1.5	—	0.4	—	"
41	12	8.0	6.0	—	Nephritis
42	11	11	3.7	—	"
43	2.5	—	0.8	—	"
44	2.3	9.7	1.5	—	"
45	2.2	2.1	0.8	—	"
46	1.8	1.8	0.7	—	"
47	1.7	1.7	1.7	—	"
48	8.7	6.7	5.3	—	" chronic
49	5.3	3.8	1.5	—	"
50	2.0	1.5	1.5	—	"
51	1.8	4.2	1.8	9.6	"
52	3.5	3.5	2.0	—	Nephrosis
53	6.0	6.7	1.8	—	Toxaemia of pregnancy
54	3.3	1.2	1.2	—	" " "
55	1.5	1.5	1.5	—	" " "
56	1.2	1.2	0.3	—	" " "
57	3.3	—	1.9	—	Papilloma of bladder
58	3.0	4.5	0	18	Peritonitis
59	16	—	5.2	—	Pyloric ulcer
60	12	—	6.6	48	Steatorrhoea
61	3.8	3.5	1.5	60	"
62	1.5	—	1.5	—	Stenosis, mitral
63	15	9.8	3.4	—	Stricture, urethral
64	2.6	1.8	1.8	—	Sprue
65	3.7	1.5	0.8	—	Hypertension
66	1.7	2.2	0.4	—	Thyrototoxicosis
67	1.3	—	0.4	—	Ulcerative colitis
68	3.8	3.8	1.1	10	Undiagnosed
69	2.7	6.0	2.0	—	"
70	4.5	—	0	—	"
71	2.7	—	0.7	11	"
72	6.0	3.0	1.5	—	Uraemia
73	2.1	1.4	1.0	17	Wilson's disease