THE HAEMOLYTIC POWER OF URINE.

THESIS SUBMITTED BY

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1927

The Haemolytic Power
of Urine.

I N D E X.

S E C T I O N 1

I N T R O D U C T I O N.

T E C H N I Q U E .

GRAPHICAL RECORDS

of

HAEMOLYTIC ACTIVITY,

of

FIFTY SPECIMENS of

URINE of UNSELECTED

PATHOLOGICAL

CONDITIONS

observed over a period of several days.

GRAPHICAL RECORDS

of

BILE ACID CONTENT OF

THESE URINES

with Oliver's Test.

SPECIFIC GRAVITY

READINGS.

S U M M A R Y.

Comparison of the haemolytic activity of fifty urines from healthy individuals.

THE HAEMOLYTIC POWER OF URINE.

INTRODUCTION.

This interesting subject, a study of the haemolytic activity of urine, on which there is little available literature, is of comparatively recent development.

If the complex nature of such a substance as urine, be taken into consideration for a moment, the complexity of any investigation such as this will be realised in so far as attempting to obtain definite facts are concerned.

Again, if urine be looked upon not as a single substance but rather as a collection of substances it is obvious that any results obtained must necessarily be interpreted with caution.

The first thing that comes to one's mind when the question of the presence of haemolysins in urine is thought of, seems to be such simple factors as, Decomposition, Tonicity, or some of the well known constituents which may occur in urine from time to time. Curiously enough these factors, as has been shown and is corroborated in the following experiments, seem to play little, if any, part in the production of haemolysis of the erythrocytes of a blood suspension.

The/

The observers who have investigated this question from various points of view are McKee (1915), Molinari (1919) and Ponder (1921). Each of these observers has worked separately and their results have been widely divergent.

Their conclusions regarding the phenomenon were as follows:-

McKee (the first investigator) stated that haemolytic activity of urine of certain morbid states is of fairly common occurrence; that it has never been observed in the urine of normal individuals and that the haemolytic agent is presumably not a normal constituent of urine.

Ponder, on the other hand, observed haemolytic activity as a regular occurrence when he examined the urines of insane persons (Individuals however whose general health was good.), when these urines were tested by a sensitive method. He found that in previous work on the subject important factors had been overlooked.

In later observations by Ponder it was shown that 64 per cent of the urine of normal individuals were haemolytic if examined at one period only.

Ponder further concluded that the haemolytic substance appeared to be a stable chemical compound capable of being extracted from the urines by various solvents and he noted that the haemolytic power of urine/

urine was inhibited by various substances, notably blood serum. His work justified the suggestion that this haemolytic activity is dependent on the urine containing minute traces of bile acids and their salts, and contrary to McKee he regarded the presence of a haemolytic substance in the urine as physiological.

The work of Molinari can be summarised as follows:- That the urine has no haemolytic action and that indeed there would appear to be an anti-haemolytic substance normally present; that this property may be used for diagnostic purposes in nephritis and cancer, the urines of which conditions he found to be less anti-haemolytic. In a word, then, the observations so far, are:-

(1) That only urines of certain morbid states are haemolytic.

(2) That the urine of normal individuals may be, and for the most part, is haemolytic.

(3) That urine, whether normal or otherwise, is not haemolytic - if anything anti-haemolytic.

Such a subject obviously will bear further investigation and the experiments described in this thesis were commenced with the idea of obtaining, if possible, some further information regarding this subject. In short:-

(1) To observe if the urines examined are haemolytic.

(2) To observe if only the urines of pathological conditions are haemolytic. For this purpose urines/

urines from unselected morbid conditions were collected and examined on consecutive days to observe any variation in activity.

(3) To observe if the urine is more haemolytic or less haemolytic in any individual condition and to obtain further specimens from other patients suffering from the same condition, to ascertain if the results are consistent.

(4) To note if any drug used in the treatment of such conditions might be excreted in a form in the urine which would influence the haemolytic power.

(5) To carry out a delicate test for bile acids in the urines observed, to ascertain if the parallelism described by Ponder between the bile acid content and the haemolytic activity is apparent. Also to observe if the specific gravity of the urine, the tonicity or the pH has any bearing on the results obtained.

(6) To find if urines of morbid conditions, although non-haemolytic at one period or periods, are necessarily always non-haemolytic.

(7) To note any common substance or substances which accelerate, retard or prevent the occurrence of haemolysis in urine.

(8) To obtain Percentage Haemolysis Curves of haemolytic urines with a view to finding the nature of the reaction.

(9)/

(9) To test the resistance of erythrocytes of various animals to urine.

(10) To observe if the haemolytic activity is altered by exercise, drinking large quantities of water, etc.

(11) To ascertain whether the surface tension of the urine is related to the haemolytic activity.

The various morbid conditions from which McKee obtained haemolytic urine are found set out in his paper and will be discussed later.

TECHNIQUE ADOPTED IN ESTIMATING THE HAEMOLYTIC ACTIVITY OF URINE OF VARIOUS PATHOLOGICAL CONDITIONS.

Each specimen of urine to be tested for its haemolytic power, was collected in the morning, just after it had been passed by the patient, in thoroughly clean and dry glass-stoppered bottles.

The specimens were immediately brought to the Physiological Department where these experiments were conducted.

From six to twelve urines were tested daily, and at the commencement urine from the same patient was used on consecutive days (i.e. a fresh sample being taken daily) in order to ascertain in what manner this haemolytic power varied, and to find if urine contained a haemolytic substance or substances at certain periods only. In every case the specimens of urine were put up in small test-tubes, (which had been carefully washed and dried in the incubator overnight) in varying dilutions with saline.

The strength of Na Cl used was 0.85 per cent.

It is known that erythrocytes immersed in a solution of lower osmotic pressure than the plasma which/

which normally bathes them, undergo lysis. In the case of human red cells (and human red cells were used in the majority of these experiments) there is no lysis at 0.8 or 0.7 per cent Na Cl, - the tonicity of which does not differ greatly from that of plasma. In 0.45 per cent sodium chloride, however, or in concentrations less than this, lysis occurs.

0.85 per cent Na Cl, therefore was a concentration which was suitable for this purpose and indeed with every daily collection of urines tested, controls were put up of 1.0 cc., 0.85 per cent. Na Cl and the same quantity of blood suspension as was used in the case of the urines.

With these controls it was invariably observed that no haemolysis was produced, indicating again that 0.85 per cent Na Cl was a safe concentration to work with.

The various dilutions of urine with this strength of Na Cl, used in the case of each individual specimen were as follows.

Urine.	Saline.
1.0 cc.	0.
0.75 cc.	0.25 cc.
0.5 cc.	0.5 cc.
0.25 cc.	0.75 cc.
0.1 cc.	0.9 cc.

i.e./

i.e. the various quantities of urine used were made up to 1.0 cc. with saline.

Both the urine and Na Cl solution were very accurately measured in 1.0 cc. pipettes, graduated in $\frac{1}{100}$ths. In cases where the urine of a particular patient was very actively haemolytic, still smaller amounts of urine were taken (e.g. 0.05 cc.; 0.025 cc.) and made up to 1.0 cc. with 0.85 per cent Na Cl.

To each of these test-tubes Blood Suspension was added - the blood suspension being prepared as follows:-

One cubic centimetre of blood was drawn from the finger of the same person and placed in some 1.5 per cent citrate in a test-tube. (A fresh supply was obtained daily.)

To this was added 0.85 per cent Na Cl and the cells were washed in the centrifuge for ten minutes. This washing was repeated twice, the supernatent fluid - serum and saline - being removed after each washing by a suction pump, care being taken that no cells were taken up.

To the thus thrice washed cells of 1.0 cc. of human blood, ten cubic centimetres of 0.85 per cent Na Cl were added. Again to this 40 cc. Na Cl solution were added to give a 0.5 per cent suspension.

It is known of course that results with cell suspensions in saline, are not by any means necessarily the same as those obtained with cells in serum or/

or plasma. It is by no means certain to what extent cells are altered by washing, and some of their properties depend on whether they are washed in a fluid which permits coagulation.

It was found in these experiments that by using a blood suspension which had been kept overnight that haemolysis was much more easily obtained, hence the necessity of a fresh suspension for each set of urines tested.

The amount of suspension added to each test tube was in most cases 1.0 cc. - and in cases where more or less has been used, the amount has been indicated.

With the quantities of urine, Na Cl solution and blood suspension mentioned above, Complete Haemolysis could be accurately estimated in all dilutions, and all readings taken indicate complete haemolysis, thus avoiding descriptive terms such as "marked haemolysis", "slight haemolysis" and the like, and also avoiding a source of error. Where the amount of haemolysis is described as a percentage, the technique is given in the same section.

All the tubes thus prepared were placed in an incubator and the time noted. The length of time of incubation and the times of reading are given.

With regard to the temperature of the incubator; this was maintained at 42° Centigrade. The reason for this, in a word, is that it has been found that at 65°C. erythrocytes will rapidly haemolyse, at 48°C. slight/

slight haemolysis occurs, while at 42°C. these are stable. 42°C is the highest safe temperature at which the experiments could be carried out. This is corroborated by the controls which were put up daily.

The urines were not filtered as it was found that complete haemolysis could quite easily be read in good natural light in unfiltered specimens. Also, as will be shown when urines containing albumen were boiled and the precipitate filtered off, no haemolysis occurred, the haemolytic substance or substances evidently not passing into the filtrate - a characteristic of many haemolysins.

The simplest data were noted regarding the patients whose urine was tested, - unless there was any peculiarity in the results obtained, - merely the diagnosis and any relevant points in the history. A note on the treatment as far as any drugs concerned, might have a bearing on the haemolytic activity if excreted in a form which would influence this.

Lastly the patient's age was noted.

The technique just described does not differ markedly from that employed by McKee, except that his quantities were measured in drops; the method employed here as has been noted is in fractions of a cubic centimetre. McKee's experiments were carried out with 15 drops of urine added to the cell suspension. His reason for this was that while he found that with some urines, 1 drop added to 1c.c. of the blood suspension induced haemolysis, he did not find that where 15 drops of urine did not cause haemol - ysis, 30 drops would. In other words that doubling the amount is without effect. This has certainly not been borne out in the following experiments save in cases where the urine contained a substance which prevented the occurrence of haemolysis, e.g.Albumen, provided this substance was present in sufficient amount. McKee states that the laking action is not purely a matter of quantity, for in testing urine which had been reacting daily he noted that 1 or 2 drops might cause laking before 10 to 15 drops. As a matter of fact at the commencement of the experiments given here something of the same nature was observed, e.g. that a tube containing let us say 0.25 c.c. urine (with 0.75 c.c. 0.85 % NaCl Sol..) would occasionally - very occasionally - show complete haemolysis apparently before the 0.5 or 0.75 dilution. As this happened so extremely infrequently it seemed rather difficult to explain. This phenomenon seemed to be explained however by the fact that the specimens used were unfiltered (for reasons/

reasons/ previously stated) and in the vast majority of cases no difficulty was experienced in reading complete haemolysis. In a few specimens,however, a cloudiness was apparent after four hours incubation and this was naturally more marked in the undiluted tube and in the lower dilutions, so that while it was obvious that complete haemolysis had taken place in the tubes containing only say 0.1 c.c. of this urine,it appeared that haemolysis was not complete when the tubes containing a greater amount of urine were shaken up. Nevertheless although this may ex- some such cases it was again noted when Percentage Haemolysis experiments were being carried out, and where probably 20 tubes were put up with the same amount of the same urine in the incubator at the same time, that occasionally all the tubes did not show the same degree of haemolysis after an identical period of incubation. Such urines were obviously unsuitable when endeavouring to obtain a percentage haemolysis curve.

McKee's investigations were carried out at a temperature of 37°C. As stated under" Technique " the temperature used here was 42°C.- the highest safe temperature that could be employed.(i.e. the highest temperature which would accelerate the reaction but yet in itself would not produce haemolysis of the erythrocytes of the suspension.) That it was a convenient temperature was shown by the daily controls in which no degree of haemolysis was ever observed.

TECHNIQUE IN BILE ACID TEST.

A test for Bile Acids was carried out on a considerable number of these urines tested for their haemolytic activity, in order to ascertain if there was a relationship between the bile acid content of urine and this activity, because bile acids are known to cause haemolysis if present in sufficient amount.

The results obtained and discussion are given later; the technique adopted was as follows.

Oliver's Test was used throughout, this test depending on the power of Bile Acids to precipitate peptone in acid solution. Pettenkofer's Test has been found to be quite unreliable in concentrations of less than 1 : 1000 Sodium Taurocholate and this concentration will effect rapid haemolysis, - the reason why Pettenkofer's Test was not employed here.

The urine was filtered until absolutely clear, acidified if necessary, and diluted if necessary until the specific gravity was less than 1008.

To sixty drops of the solution:-

Powdered Peptone (Witte's)		drachm ½
Salicylic Acid		grains 4
Acetic Acid		drachm ½
Distilled Water	to	oz 8.

(Filtered repeatedly until transparent)

in a thoroughly clean test tube, was added to 20 drops of the filtered urine. If bile acids be present a /

a milkiness appears. It may disappear on agitation but reappears on adding more of the solution. The test is said to be extremely delicate and nothing as yet found in the urine interferes with it. As the precipitate referred to above is in proportion to the amount of bile acids present, the test was thought suitable for the purpose of observing a parallelism, if any, between the degree of haemolysis produced by the urine and the bile acid content of that urine.

A series of tubes were made up with powdered tragacanth, methylated spirit and water, in varying proportions, and thus varying degrees of opacity were obtained. These were numbered 1 to 9 and the precipitate in the bile acid test read from them. There was no attempt to find the amount of bile acids present, but the method was sufficiently accurate for the purpose of observing any relationship between the Bile Acid content of the urine and its activity in the haemolysis of the blood suspension. This method also enabled the results so obtained to be given in the form of graphs, rendering comparison more accurate.

The first series of the following graphs are only records of the least quantity of the urines tested required to produce complete haemolysis of 1.0 cubic centimetre of the human blood suspension already described, after incubation at 42 C. for four hours.

They serve to show the periodicity of the occurrence of this phenomenon with regard to the haemolytic substance or substances found in the urine.

The records are, in the first series, taken over a period of four consecutive days which emphas - izes the marked variation in the haemolytic activity of urine from day to day. Records taken on consecutive days also suggests the fallacy of calling a urine or urines non-haemolytic which have only been observed on one day.

There was no other reason than convenience for observing these specimens for this period of

four days, and indeed if no haemolysis had been produced within this time, the experiments were continued with that urine until haemolysis did occur or until some explanation or conclusion was arrived at as to why the phenomenon should be absent.

Regarding the graphs themselves these have been accurately drawn from graph paper in millimetre, half, and one centimetre squares.

The Ordinate represents the highest dilution with 0.85 % NaCl at which complete haemolysis took place, (i.e.,the lowest amount of the urine re - quired.) For example 0.25 c.c. indicates that haemolysis occurred with 0.25 c.c. of that urine and 0.75 c.c. 0.85 % NaCl - (the various amounts being made up to 1.0 c.c. with the NaCl solution.)

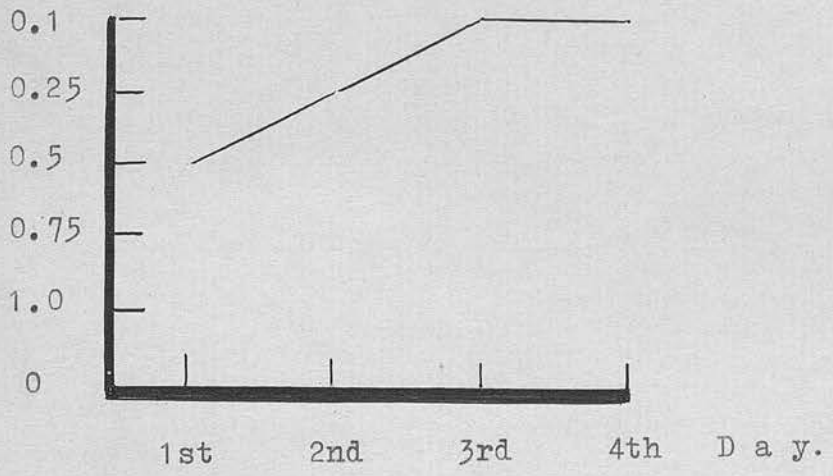
The Abscissa represents the days on which the urine was tested.

Controls were put up daily of 1.0 c.c. 0.85 % NaCl and 1.0 cc. suspension. These were invariably negative after 4 hours incubation at 42 C.

Case No. 1

G.- Female. Obesity. Age 43.
Drugs. Mag. Sulph. 3j mane.

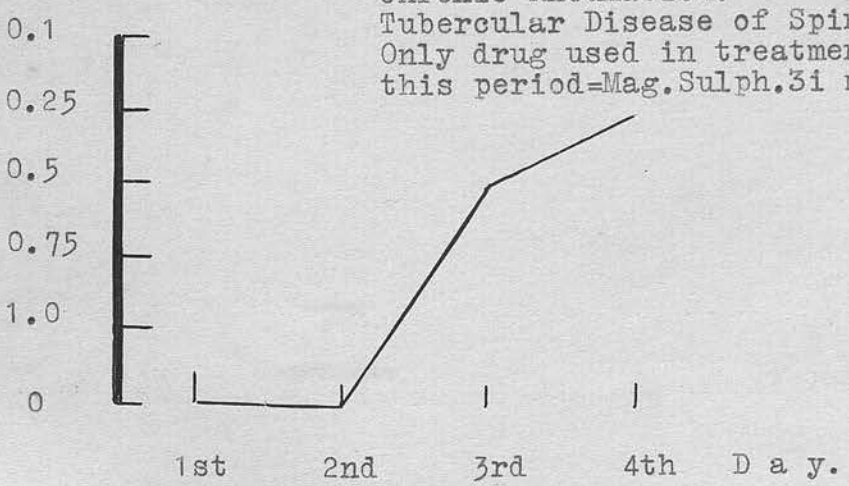
Haemolytic Activity of Urine.



A comparatively active urine.

Case No. 2

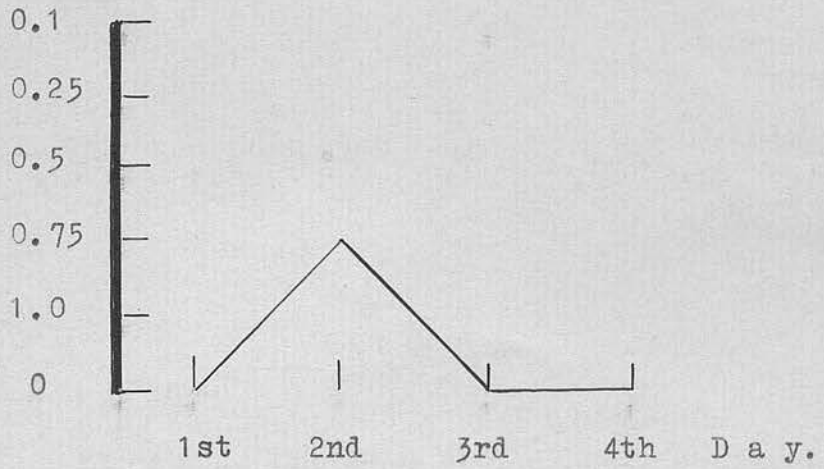
P.- Female, Aged 39.
Chronic Rheumatism.
Tubercular Disease of Spine.
Only drug used in treatment during this period=Mag.Sulph.3i mane.



This specimen is not so active; indeed no haemolysis has taken place for the first two days, although the urine con contained no abnormal constituent which would prevent haemolysis. Had it only been examined on these days it might have been classed as

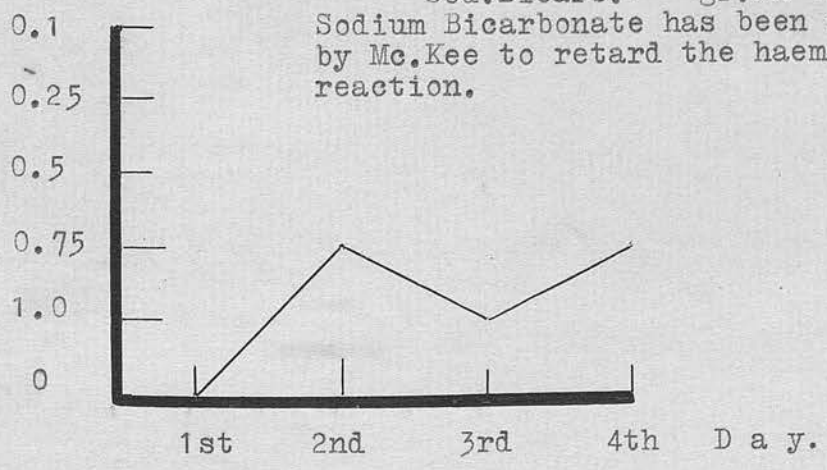
as/ non - haemolytic.

Case No. 3. S.- Female, Age 61.
Gastric Ulcer.
Acetone present in urine which may account for the decrease in haemolytic activity to some extent.



A urine very similar to the previous specimen.

Case No. 4. M.- Age 54. Mitral Stenosis following Acute Rheumatism.
Drugs=Sod.Salicylat. gr.XX t.i.d.
Sod.Bicarb. gr.XX t.i.d.
Sodium Bicarbonate has been stated by Mc.Kee to retard the haemolytic reaction.



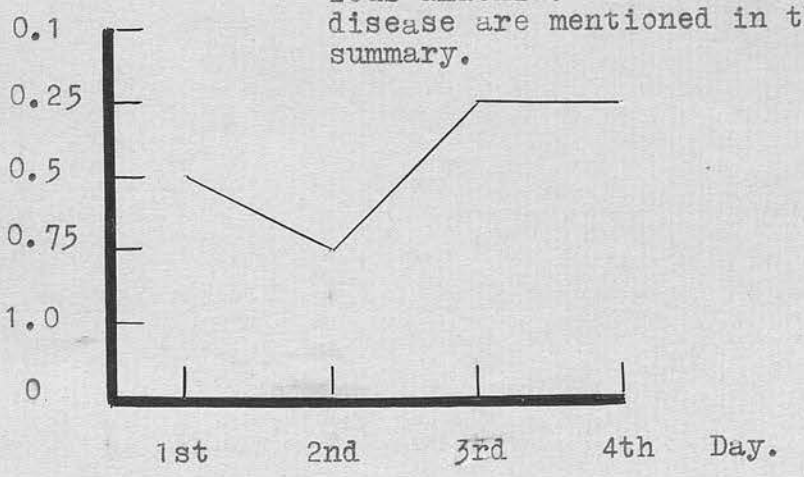
A moderately active urine only. Complete haemolysis never takes place in a dilution lower than 0.75c.c.

Case No. 5. Mc.C-. Age 31. Chronic Parenchymatous Nephritis.

No haemolysis occurred in this urine after being tested for ten days, the reason being its albumen content which varied from 7 to 9 grammes per litre. It is described later with other specimens containing substances which retard or prevent the the occurrence of haemolysis.

Case No. 6.

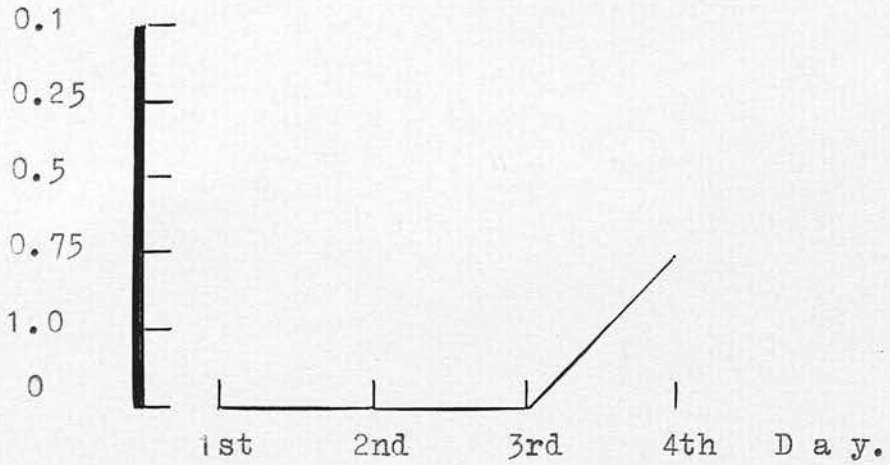
C.-. Age 26. Pernicious Anaemia. Drugs= Liq.Arsenicalis m iij t.i.d. There is no falling off in haemolytic power in this case of pernicious anaemia. Other cases of this disease are mentioned in the summary.



A comparatively active specimen; complete haemolysis takes place at 0.25 c.c. of urine, the periodicity still being shown.

Case No.7 I.M-. Age 27. Female.
Exophthalmic Goitre.
Auricular Fibrillation.
Drugs = Tinct. Digitalis mX t.i.d.

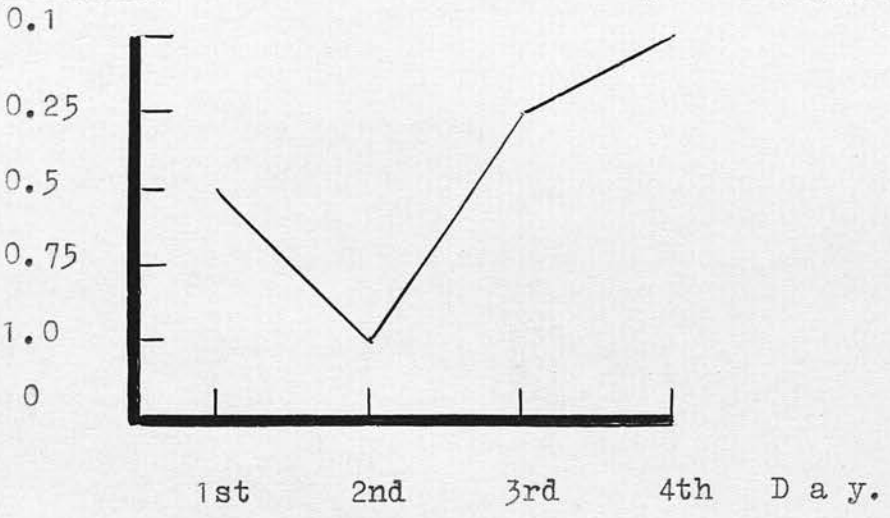
No abnormal constituents were present. Is it possible that the drug or the disease is responsible for the inactivity or is this in keeping with other urines? This urine is again examined underNo.



Very inactive. No haemolysis on three out of four days and then only at a dilution of 0.75 c.c.

Might have been put down as non - haemolytic.

No abnormal constituents present.



Comparatively active ; very active on 4th day.

Case No. 8 A.T-. Age 23. Female.
Exophthalmic Goitre.

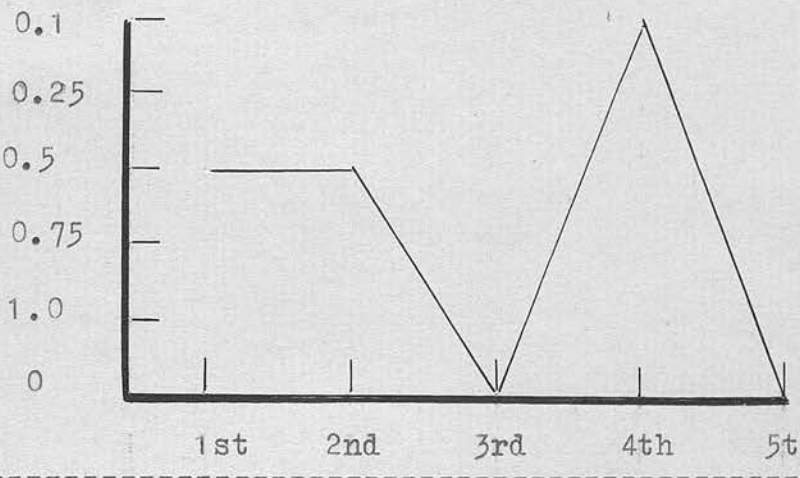
The next series of graphs are the same as those in the first series, with the addition however, of the readings obtained from the Bile Acid Test for urine. (Oliver's.) It has been possible to convert these into graphical form by comparison of the amount of opalescence obtained by the above test, due to the presence of bile acids contained in the specimens, with the set of standard tubes specially prepared.

When it is pointed out that the graduations of these standard tubes were necessarily rather fine,(e.g., No. 2 on the scale was really a very slight opalescence) it will be understood that considerable care was necessary in the readings, and it will be realised that there is in the great majority of cases no great variation in the amount contained.

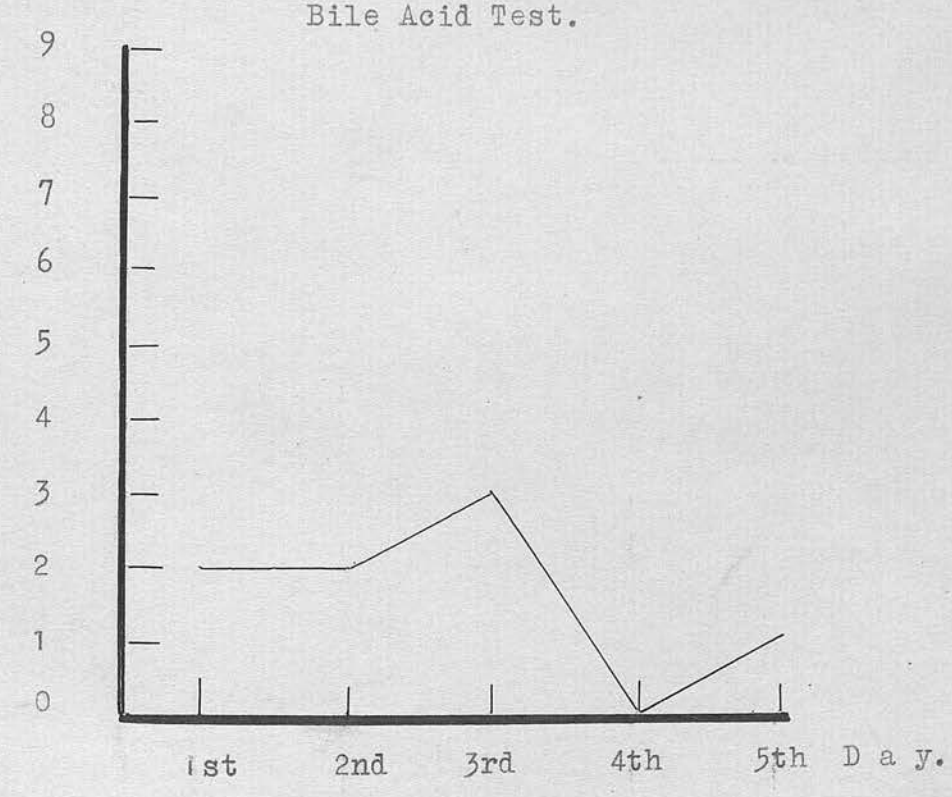
The controls run in conjunction with this series were again negative without exception.

Haemolytic Activity.

Case No. 9. T.McD-. Age 49. Male
Duodenal Ulcer.

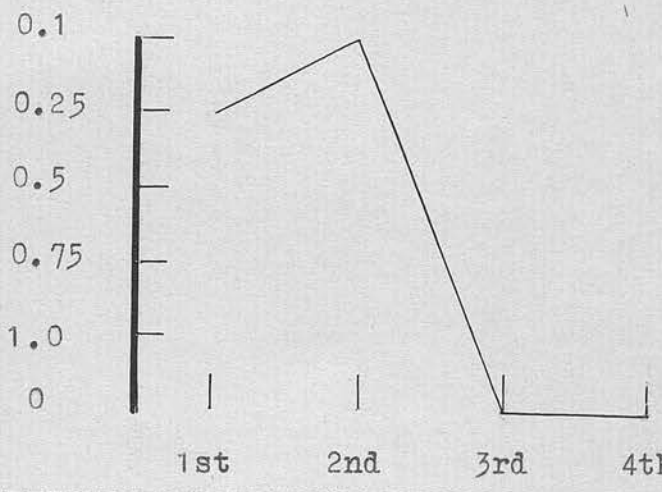


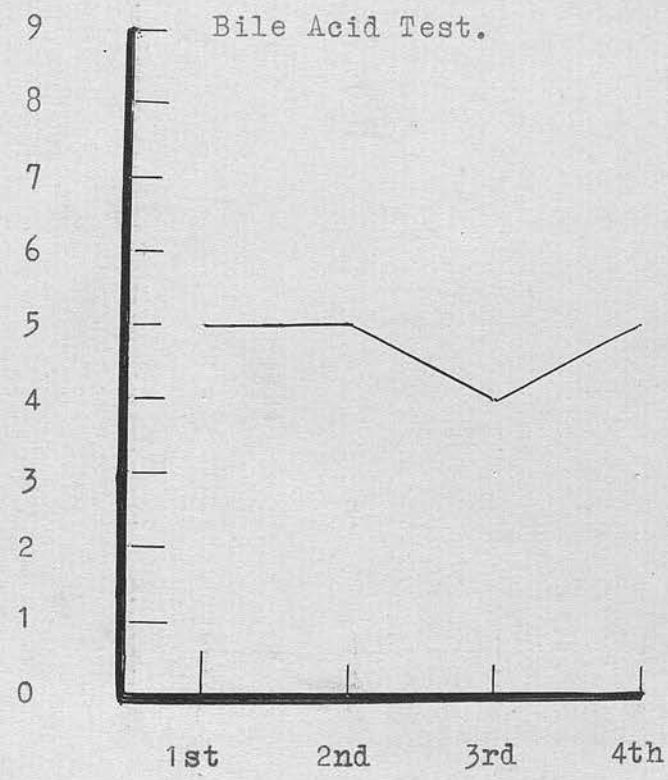
Bile Acid Test.



There is no agreement in these, in fact the Bile acid graph is contrary to the other. No bile acids were present on the 4th day by the test and yet this is the most active day of the urine.

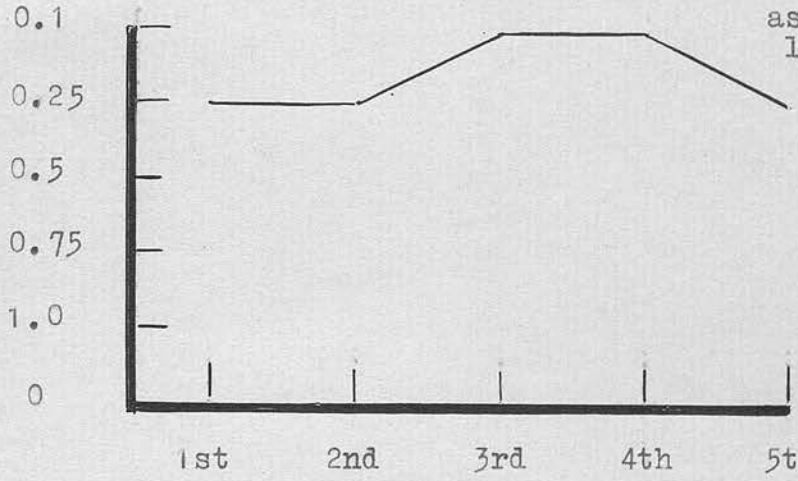
Case No. 10. F-. Age 59. Female.
Rheumatoid Arthritis.
Pericarditis.
Drugs= Ac.Acetyl Salicyl. gr X t.i.d.

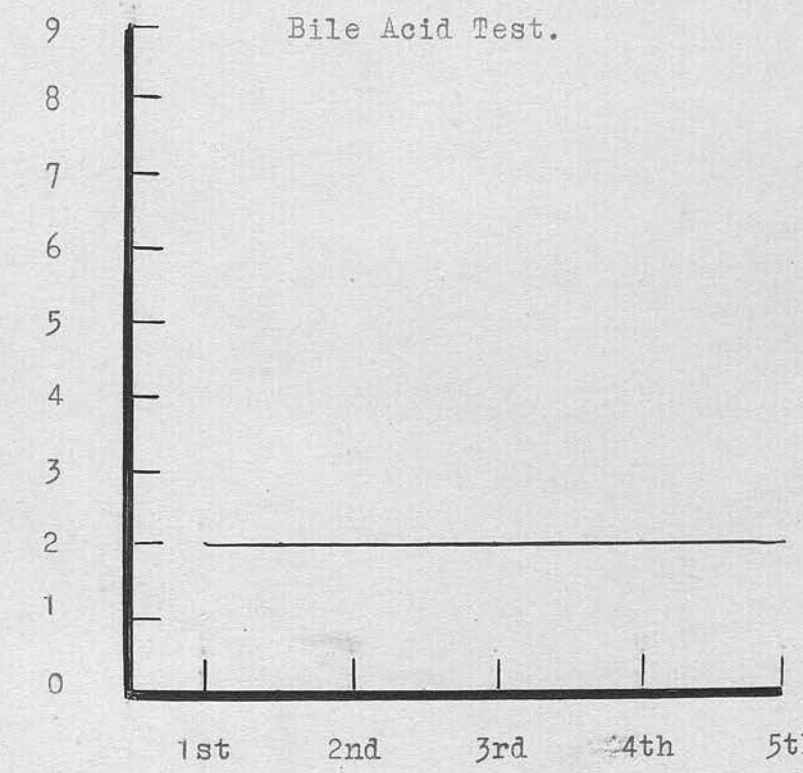




An active urine on the first two days and also a relatively high bile acid content on these days, but no corresponding fall with the decreased activity of the urine on third & fourth days.

Case No. 11. M.S-. Nephritis. This urine contained
Age 61. Blood and Albumen (fr.2 - 4 G/litre.) .
Male. and is the only urine which caused haem-
olysis with these abnormal constituents.
The contained lytic substance must have
been extremely active, and must have in
contact with the cells before the albumen
as will be later shown.



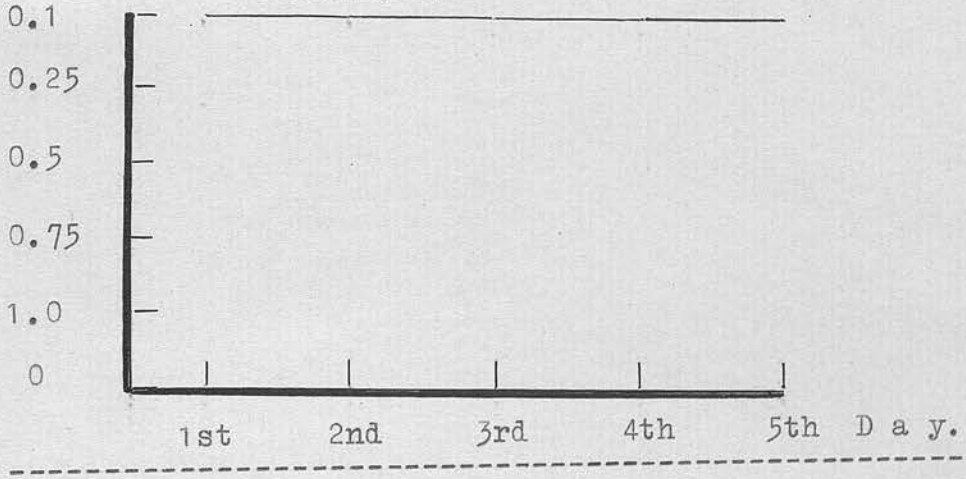


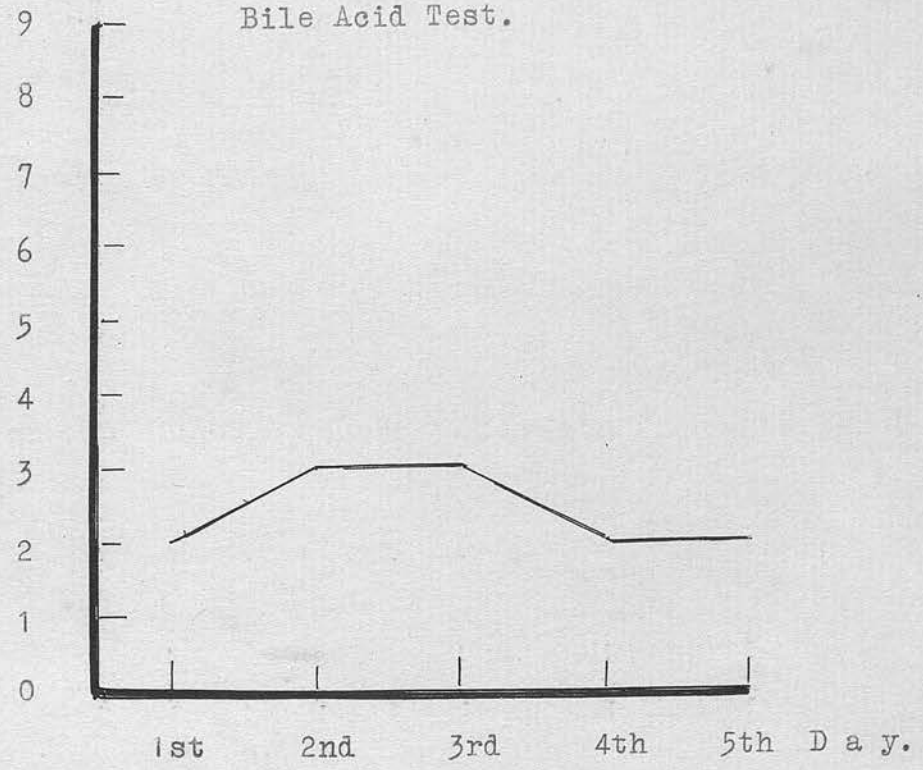
There is really no relation between these,

because it is observed that the bile acid reading

is persistently low, the urine being rather active.

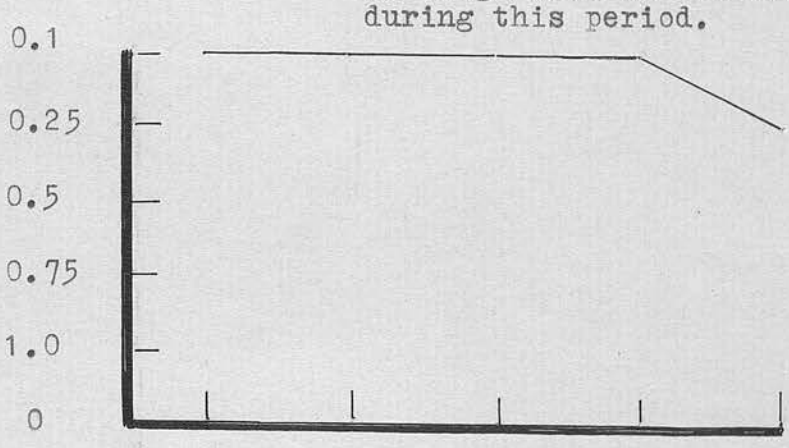
Case No. 12. D.McG-. Male.
Acute Rheumatism.
Liq. Strychninae m X t.i.d.
Iodolysin. One injection given during the period that the urine was examined.

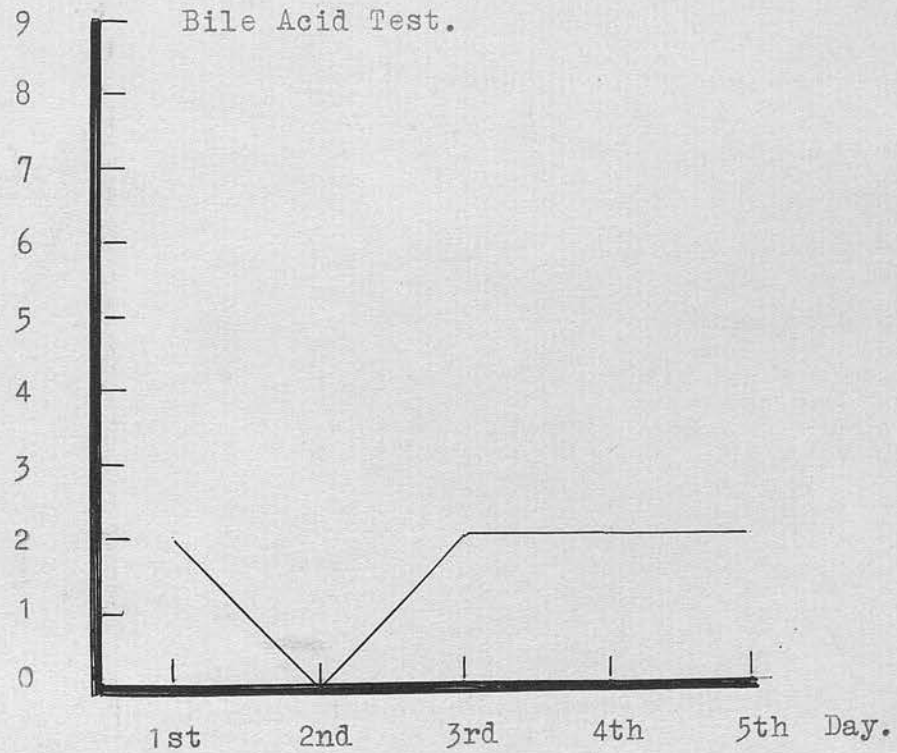




A very active urine but not a correspondingly high bile acid content.

Case No. 13. J.A-. Male.
Age 45.
Pulmonary Tuberculosis.
No Drugs used in treatment
during this period.

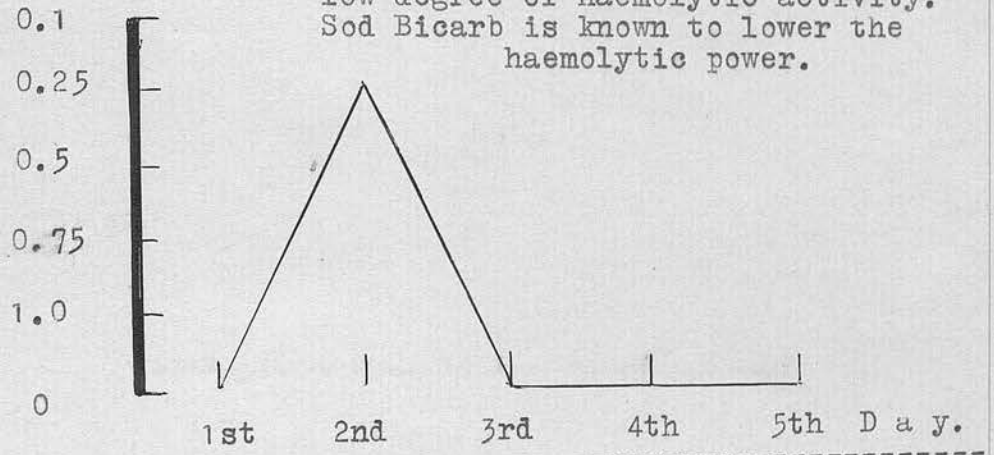


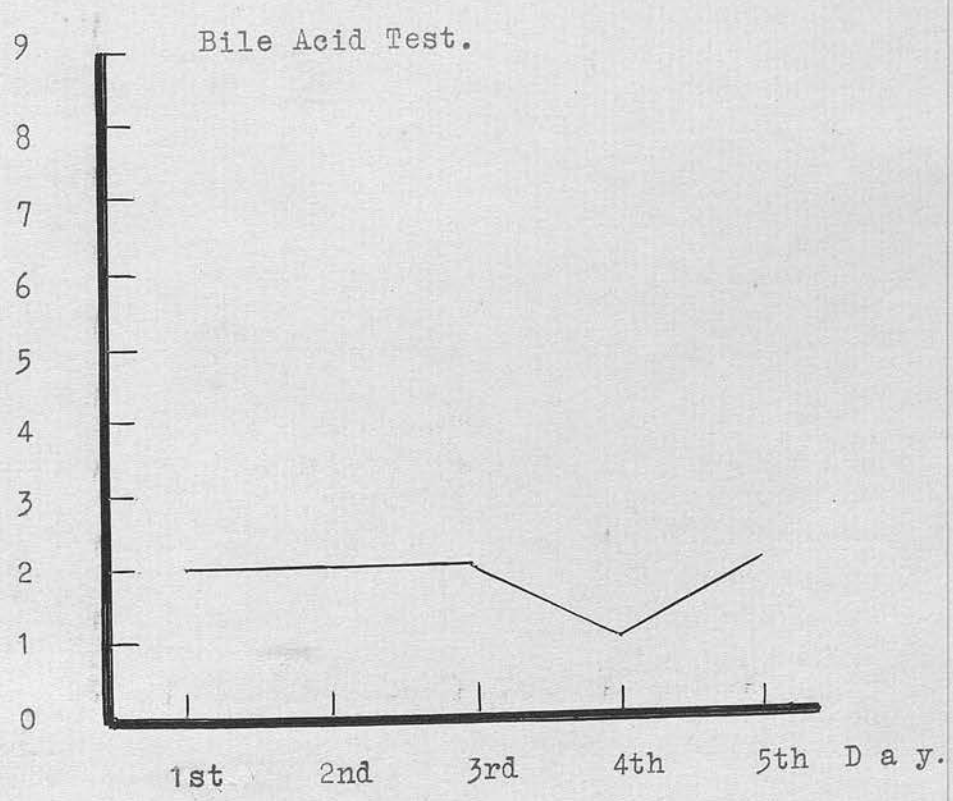


There is no relationship whatsoever in these two records.

Case No. 14.

G.D-. Male. Age 67.
Gastric Ulcer.
Haematemesis.
Sippey Diet. The Sod.Bicarb. used in the treatment may account for the low degree of haemolytic activity. Sod Bicarb is known to lower the haemolytic power.

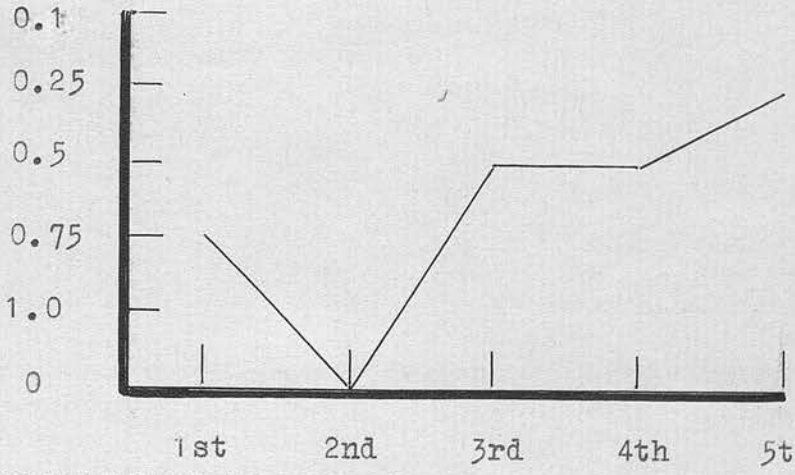


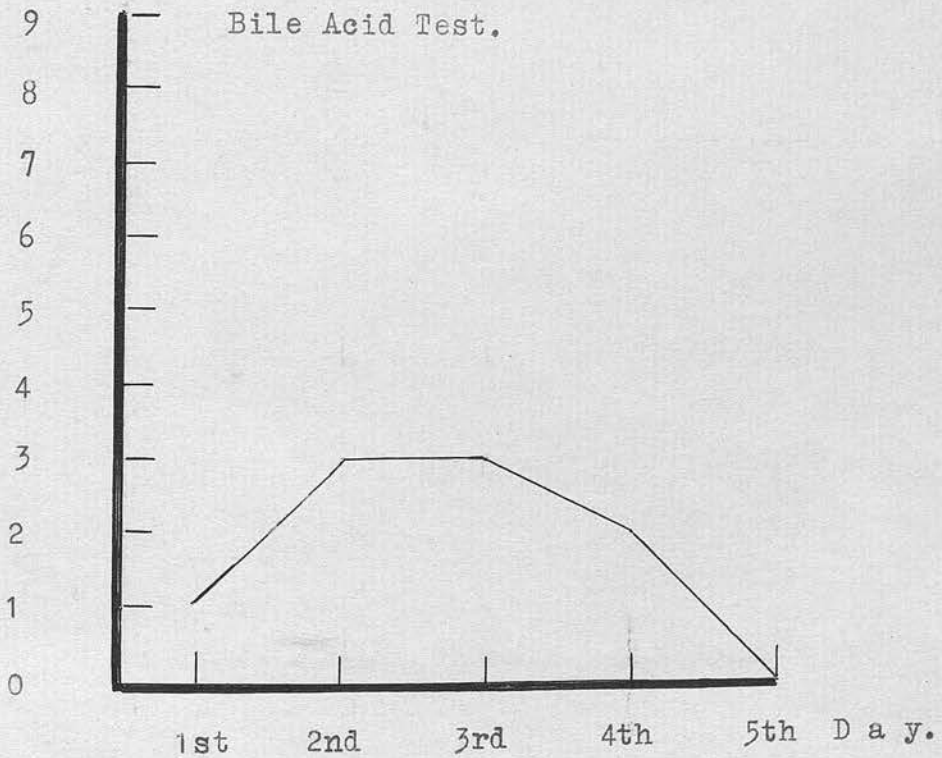


These do not coincide. There is no increase in bile acid reading to correspond with the marked haemolytic activity on the second day.

Haemolytic Activity.

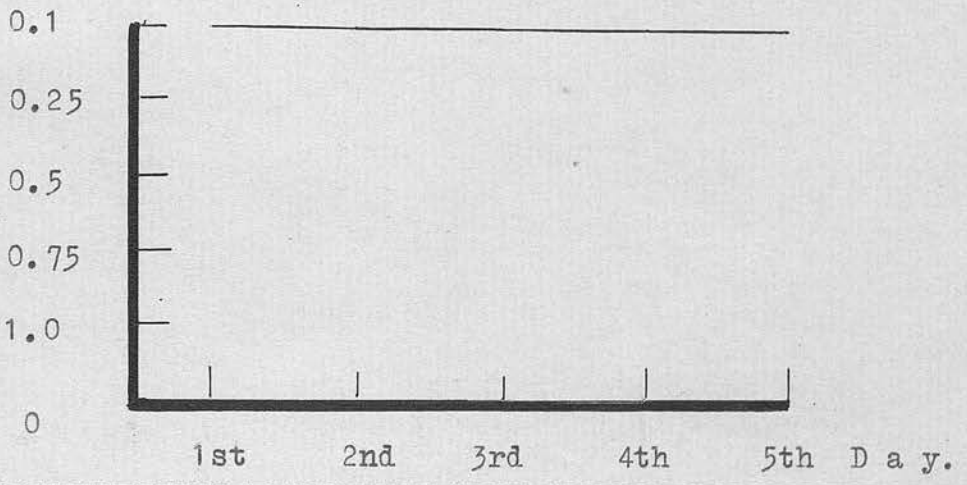
Case No. 15. D-. Female Age 62.
Chronic Cholicystitis.
No Drugs used in treatment.

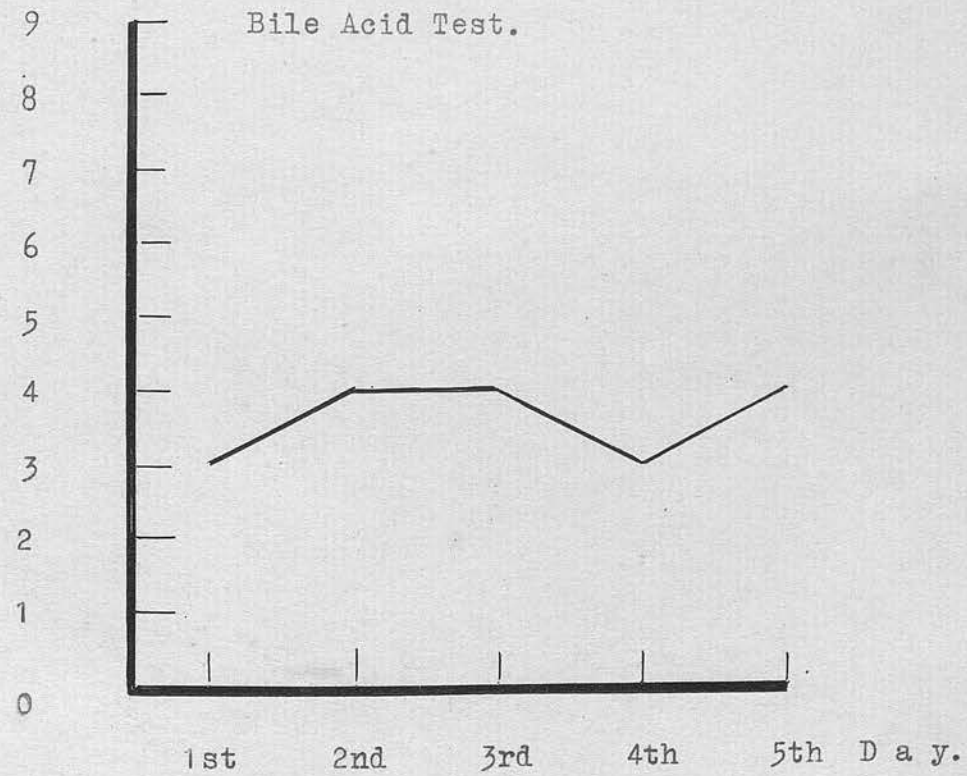




Another graph showing the bile acid readings running almost exactly opposite to those of the haemolytic activity of the urine.

Case No. 16. C-. Age 57. Female.
Malignant disease of Pelvic Colon.
Drugs= Liq.Arsenicalis m iij t.i.d.

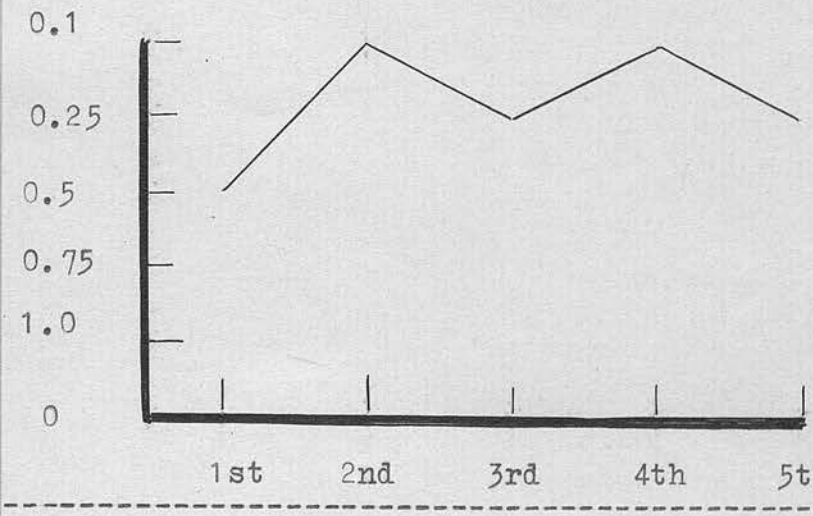


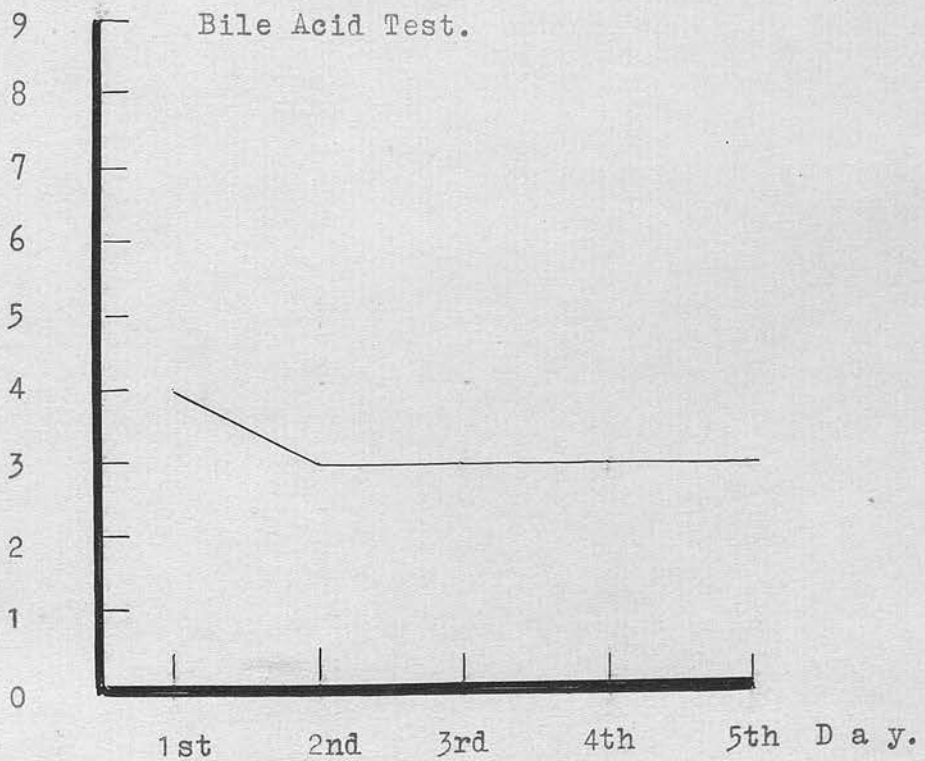


Another very active urine and also a comparatively high bile acid content but a definite agreement between the two is not shown.

Haemolytic Activity.

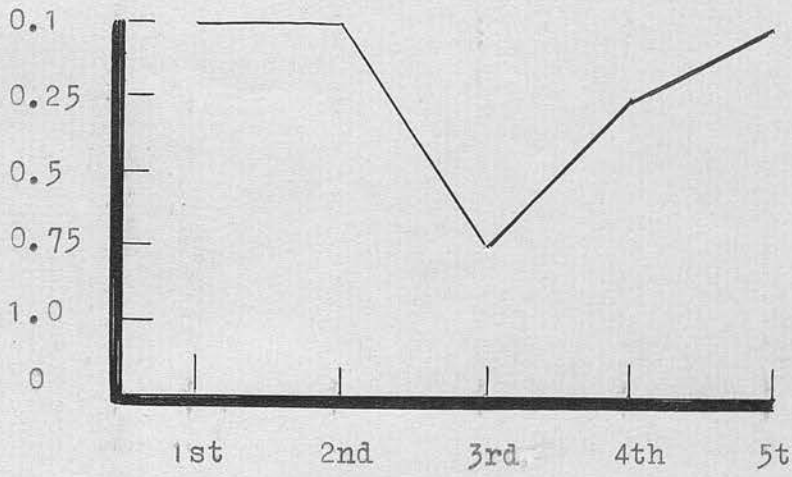
Case No. 17. A.T-. Female.
AGE 23.
Exophthalmic Goitre.



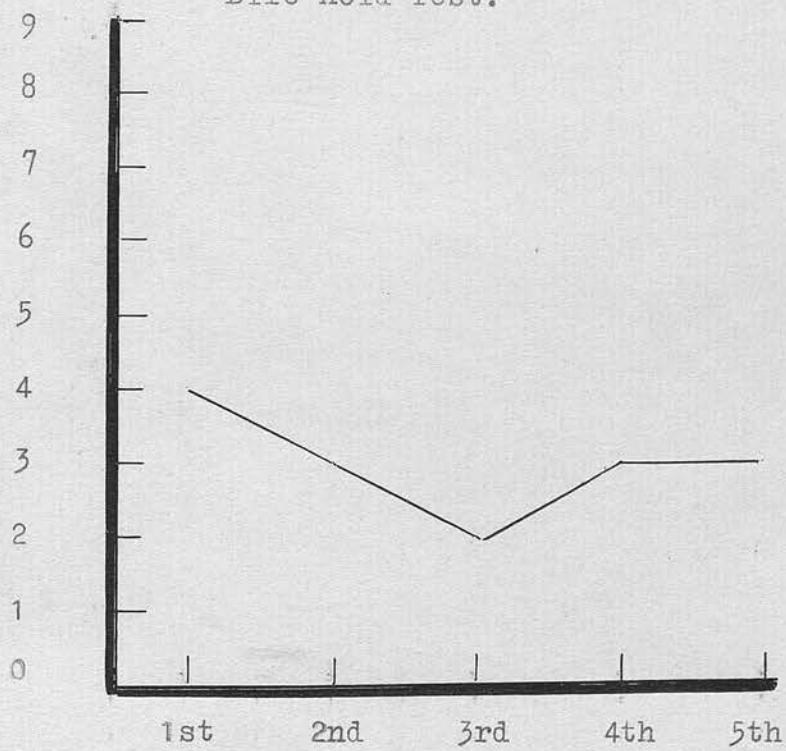


No corresponding increase in the bile acid reading when the haemolytic activity shows an increase.

Case No. 18. C.B-. Age 32. Female.
Acute Rheumatism.
Drugs= Ac.Acetyl Salicyl. gr X t.i.d.
Mag.Sulph. 3i mane.



Bile Acid Test.

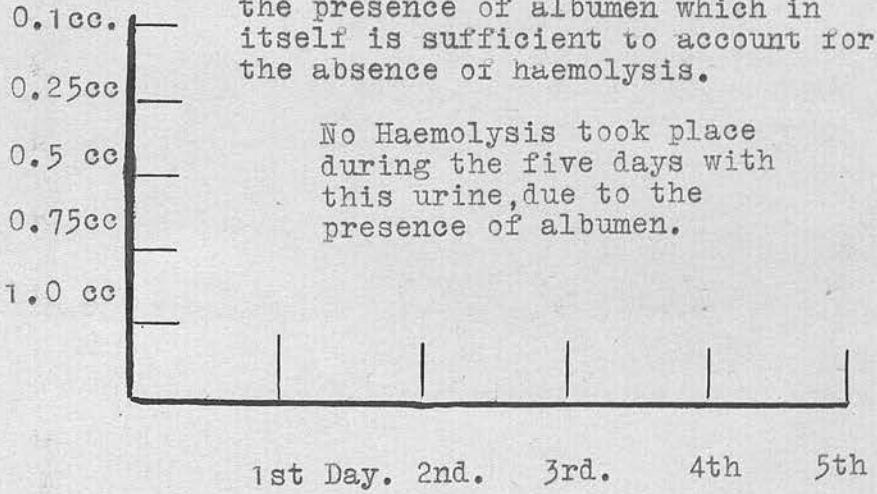


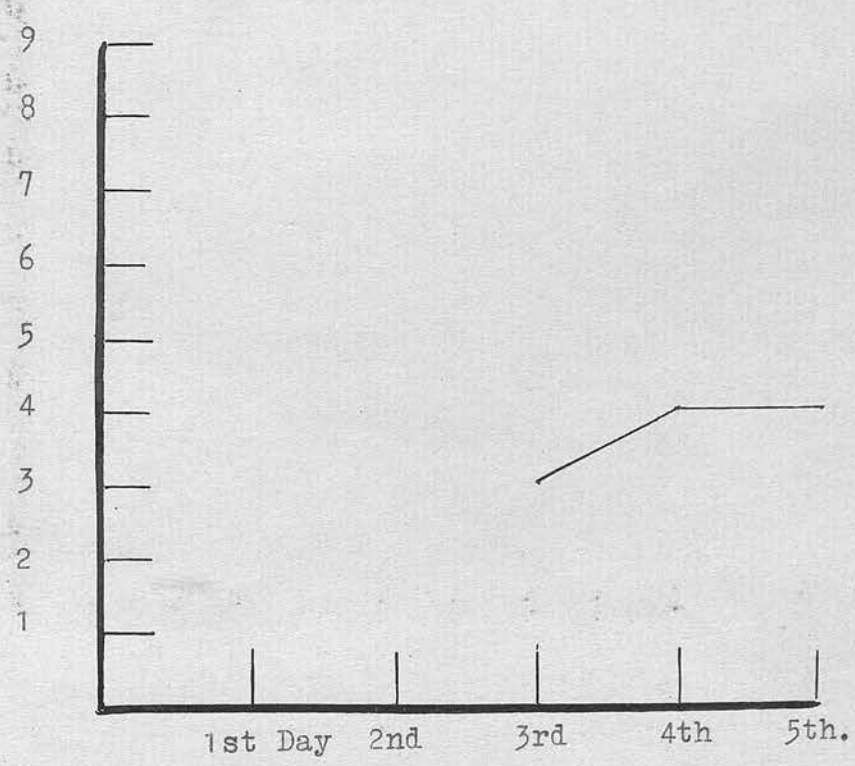
This shows some parallelism between the haemolytic activity and the bile acid content - one of the very few records which does.

Case No. 19.C.T. Pyelitis. Ureteric Calculus.

Urine contains Albumen.
Pus Cells.
B.Coli.

Sod.Bicarb was given in the treatment in large doses but the inhibitory effect is masked by the presence of albumen which in itself is sufficient to account for the absence of haemolysis.





Representing graphically the amount of Bile Acids present in the urine by Oliver's Test, comparison being made with standard tubes.

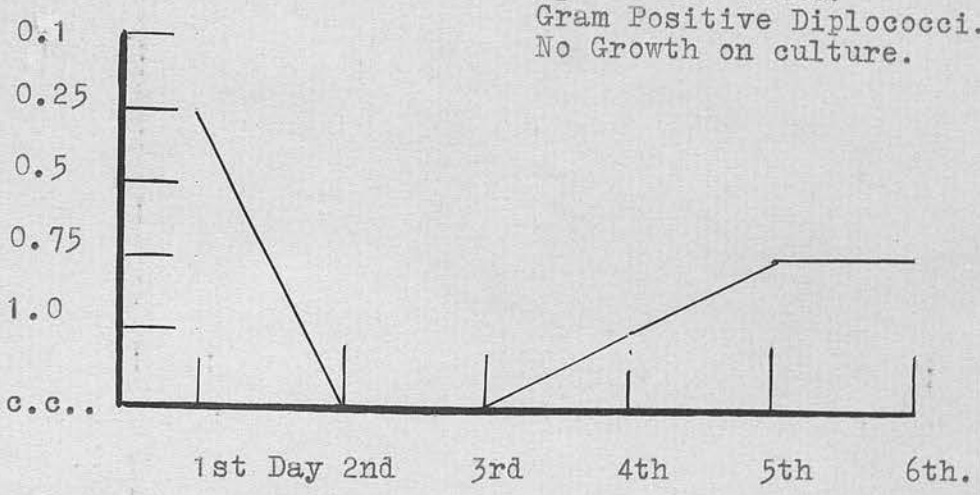
Case No. 20. **Female. Cystitis.**

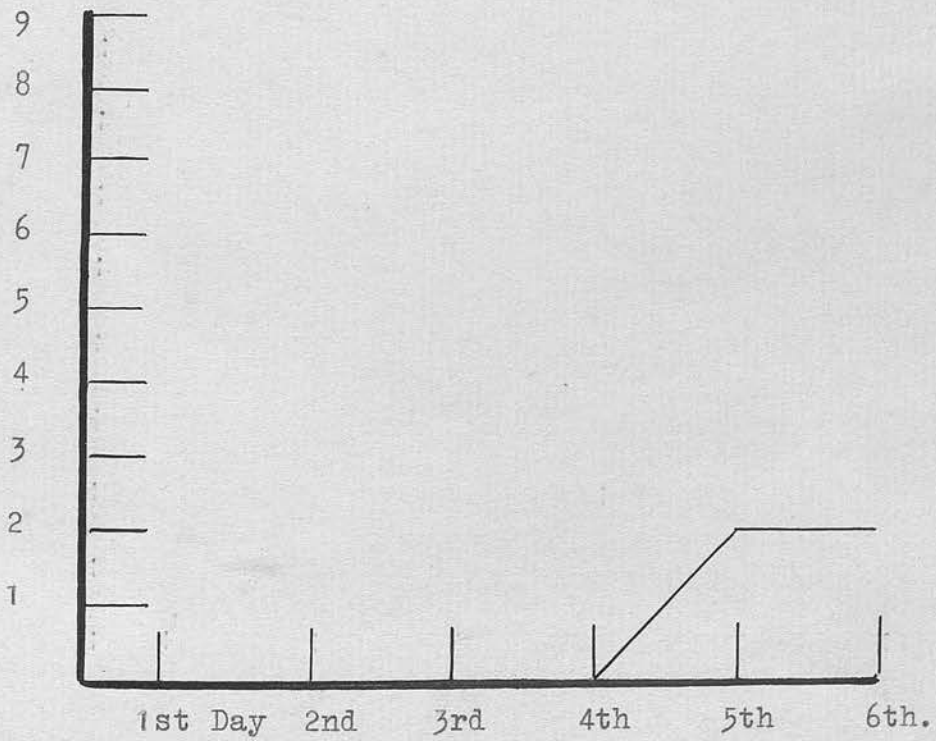
No drug treatment during this period.

B.S. Age 24.

Catheter Specimen.

Urine contains Epithelial Cells.
Gram Positive Diplococci.
No Growth on culture.

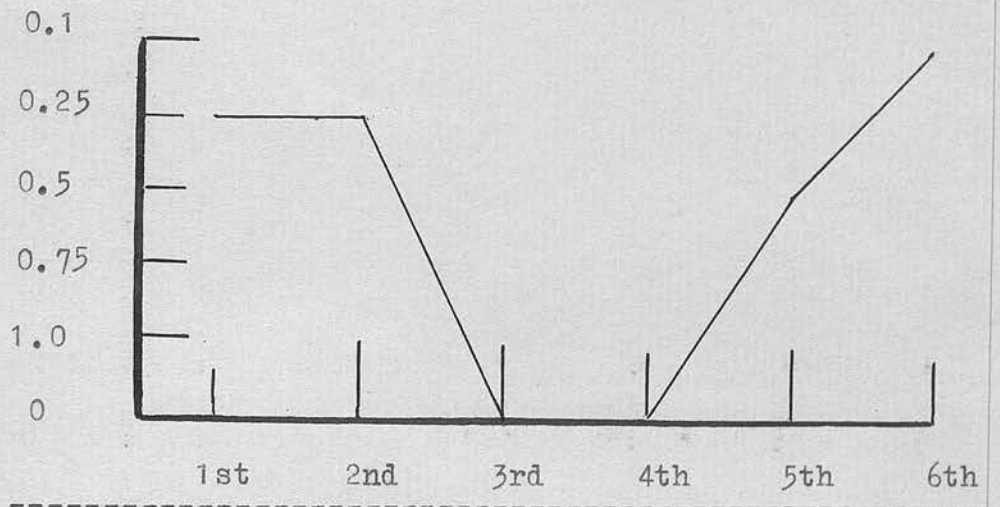




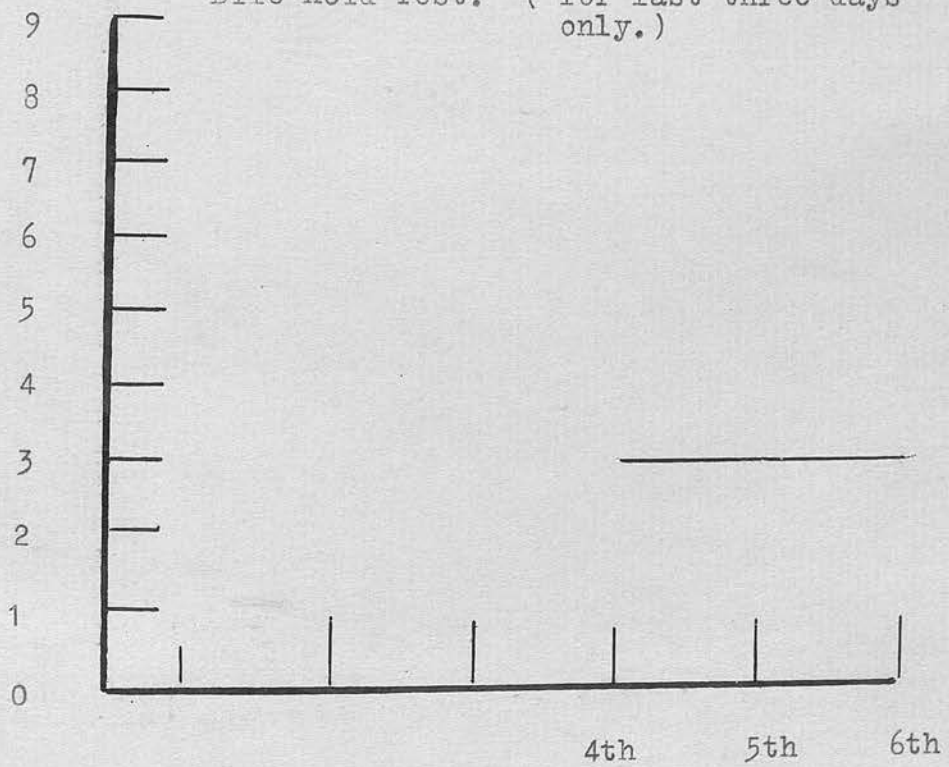
Not a very actively haemolytic specimen. The amount of bile acids present has only been observed during the latter three days in the first twelve specimens.

Haemolytic Activity. (for six consecutive days.)

Case No. 21. M.F-. Female.
Age 27.
Exophthalmic Goitre.
No drugs given at a time which would affect the specimen of urine.



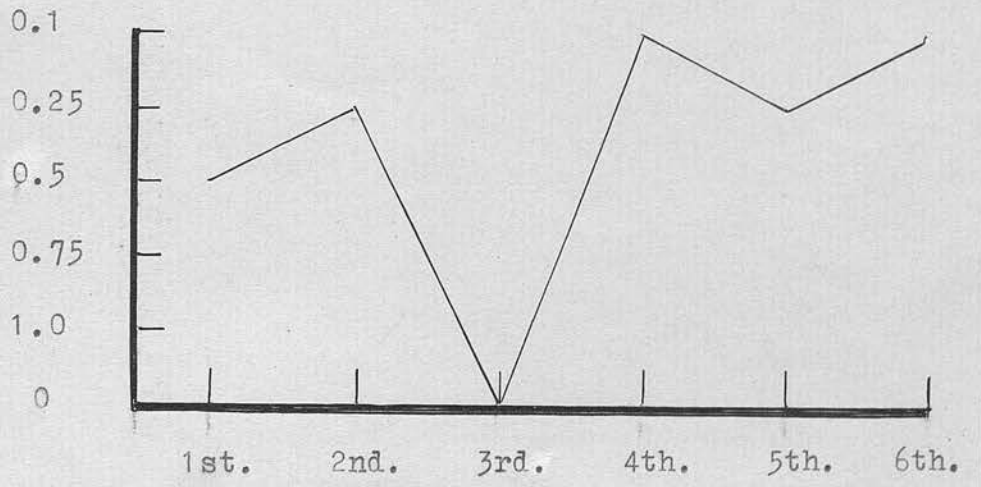
Bile Acid Test. (for last three days only.)

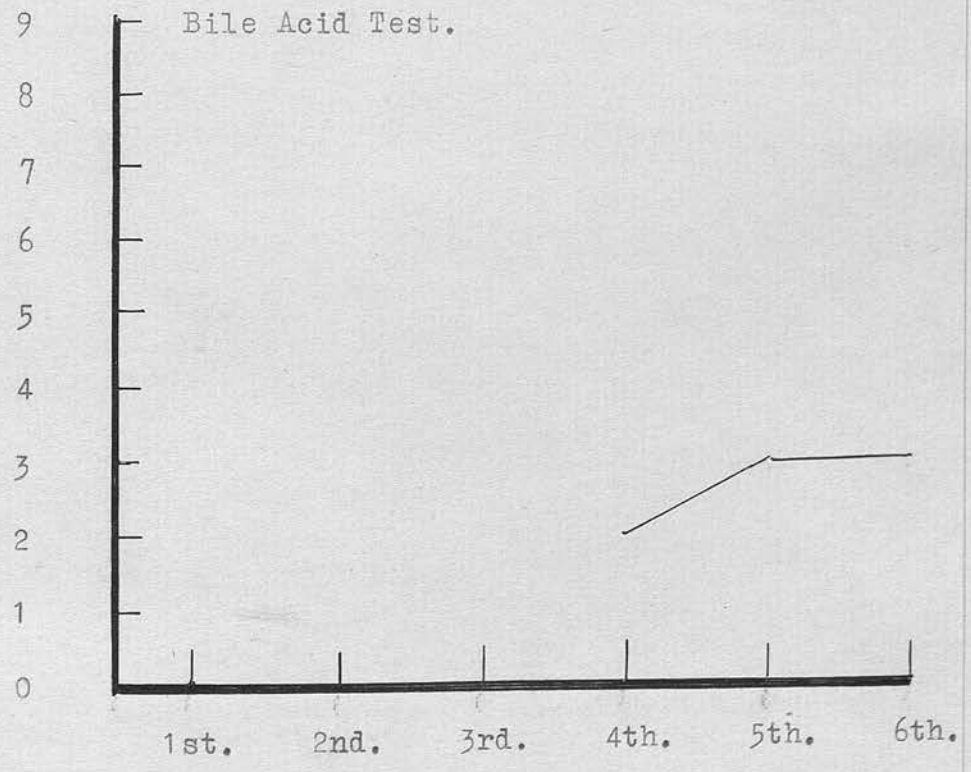


Haemolytic activity of this urine increases markedly from 4th to 6th day; no such increase in the lower graph is seen.

Haemolytic Activity.

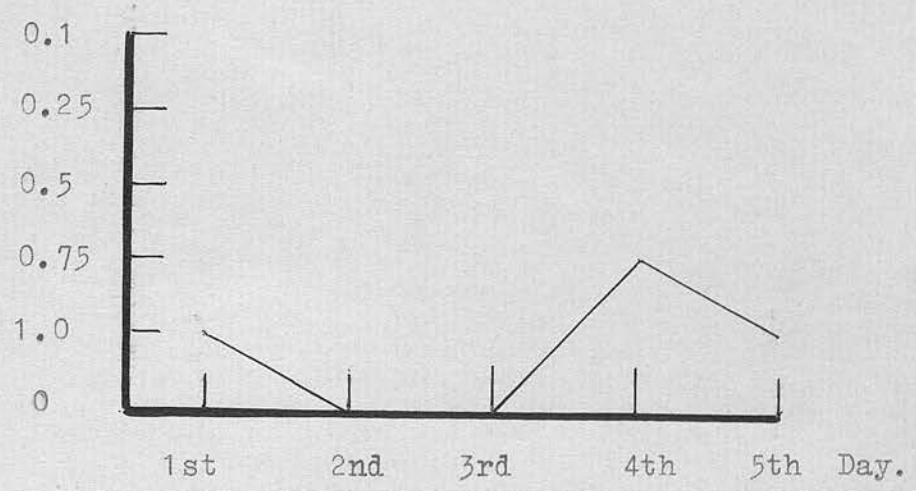
Case No.22. E.L-. Female. Age 22.
Early Disseminated Sclerosis.

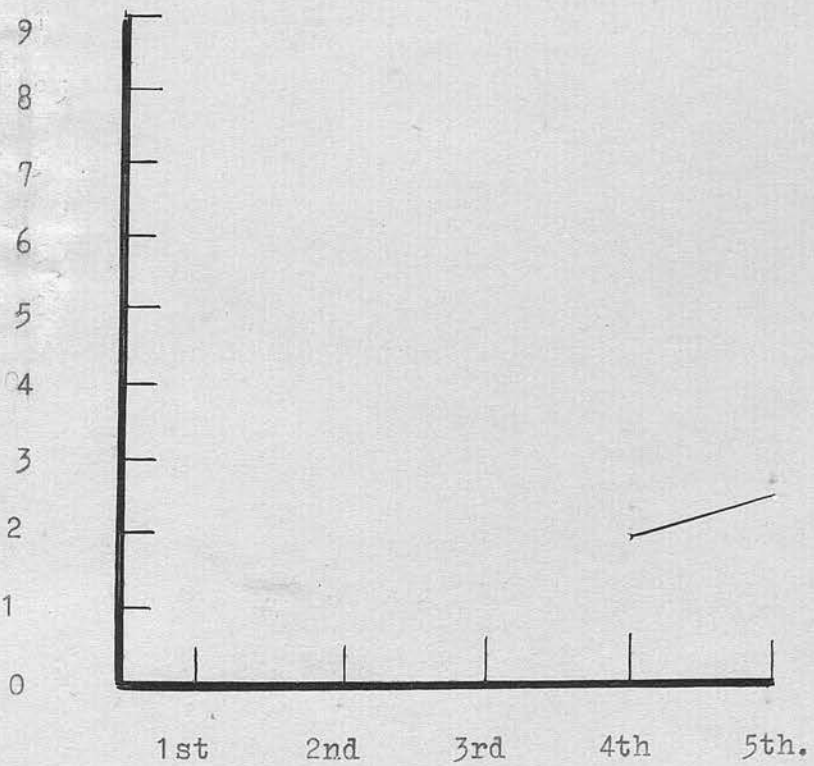




This again shows the marked daily difference which may occur in the haemolytic activity of urine. The bile acid readings do not run parallel.

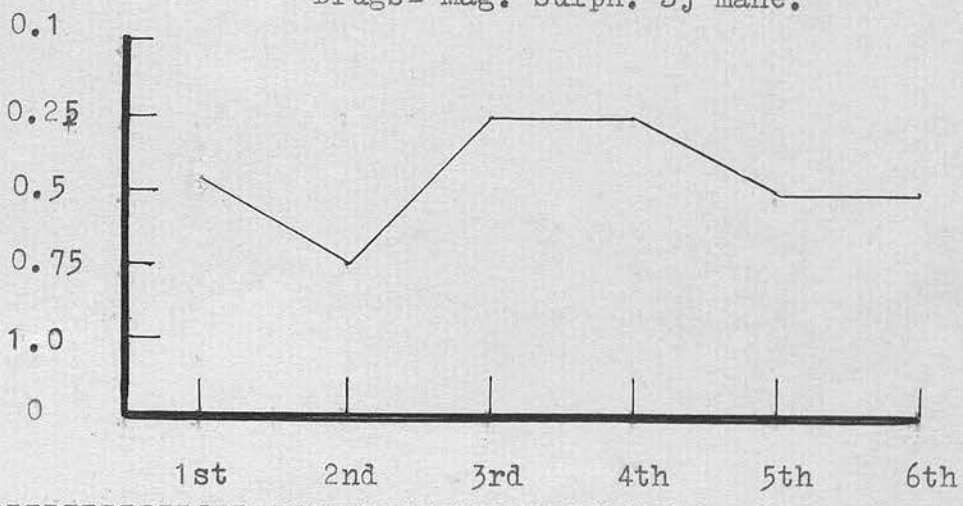
Case No.23 M-. Female. Age 24.
Pneumonia.
Resolution Stage.
No drugs given at this time.

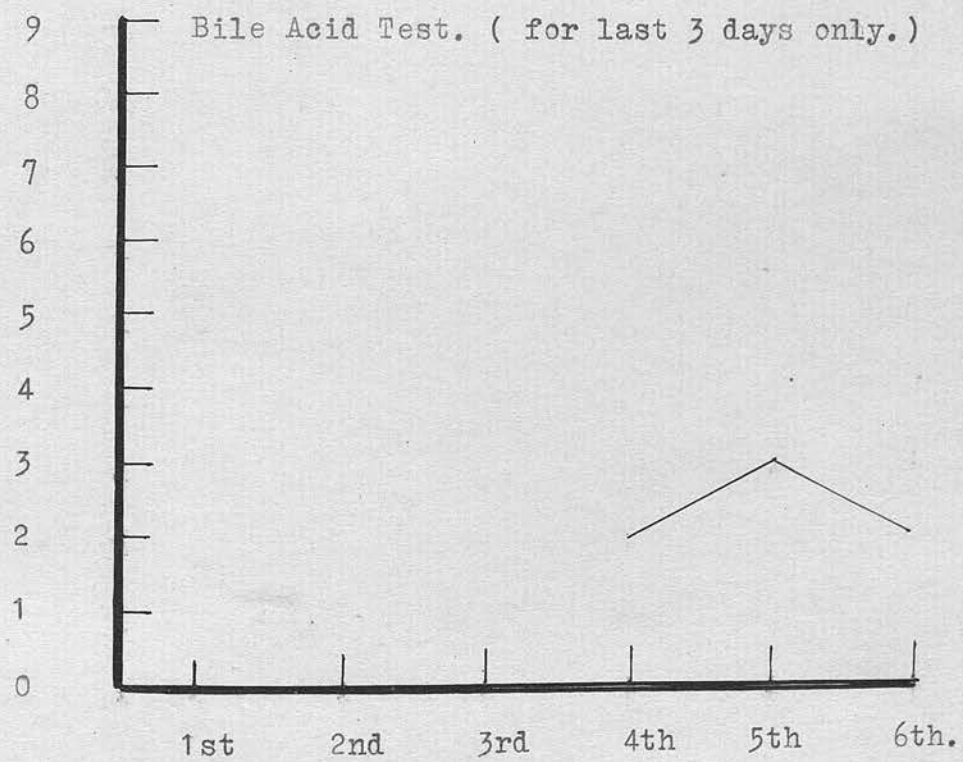




Not an active urine. Haemolysis fails to occur on two out of the five days tested, and does not occur with less than 0.75 c.c. of urine.

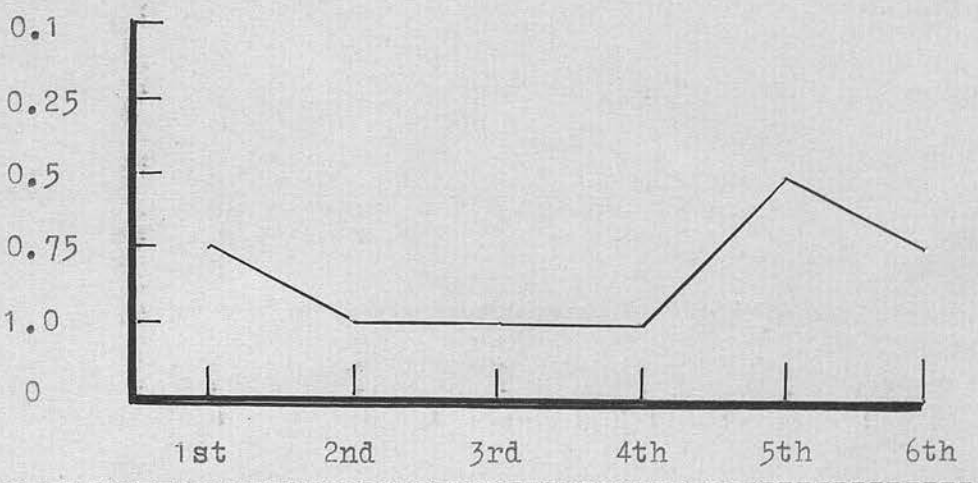
Case No. 24 E.G-. Age 48.
Rheumatoid Arthritis.
Pulmonary Infarction.
Haemoptysis.
Drugs= Mag. Sulph. 3j mane.

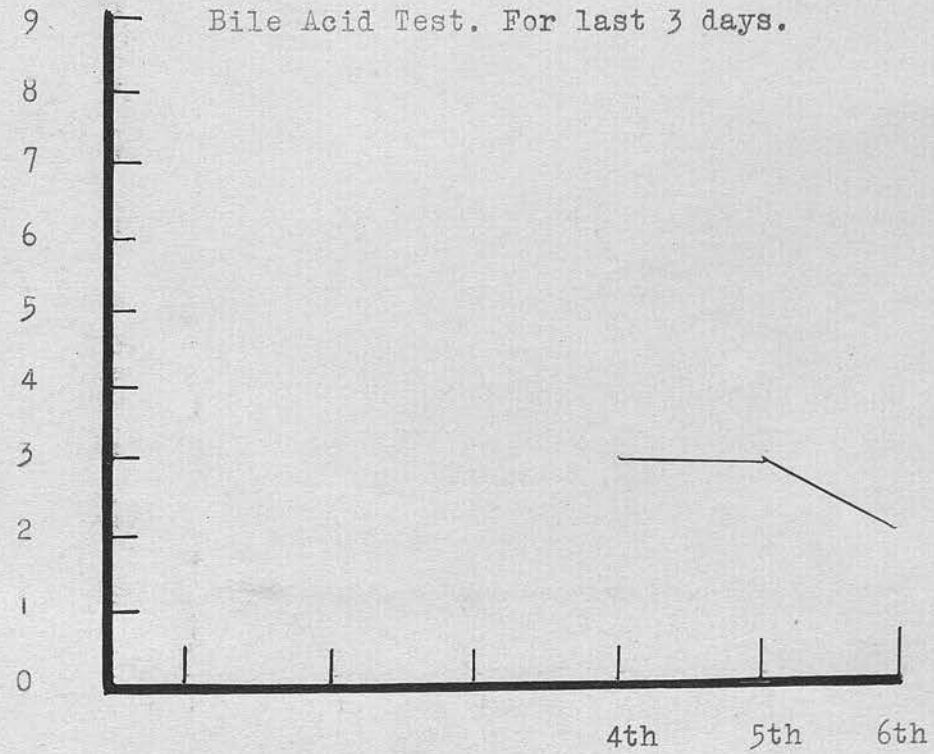




A moderately active urine., but no agreement in the bile acid reading on the days when this was carried out .

Case No. 25. J.H-. Age 6.
Chronic Bronchitis.
2 yrs. duration.
Drugs = Ammon. Carb. t.i.d.
Vin Ipecac.

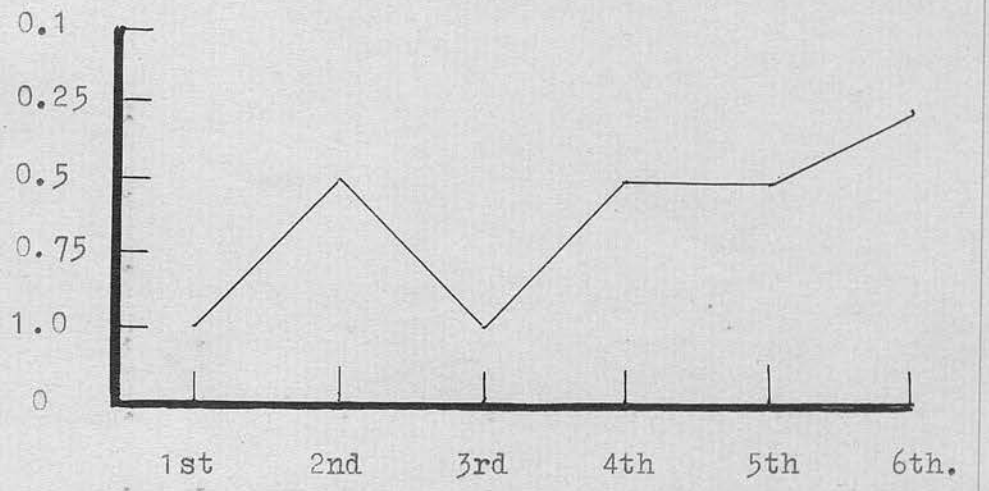


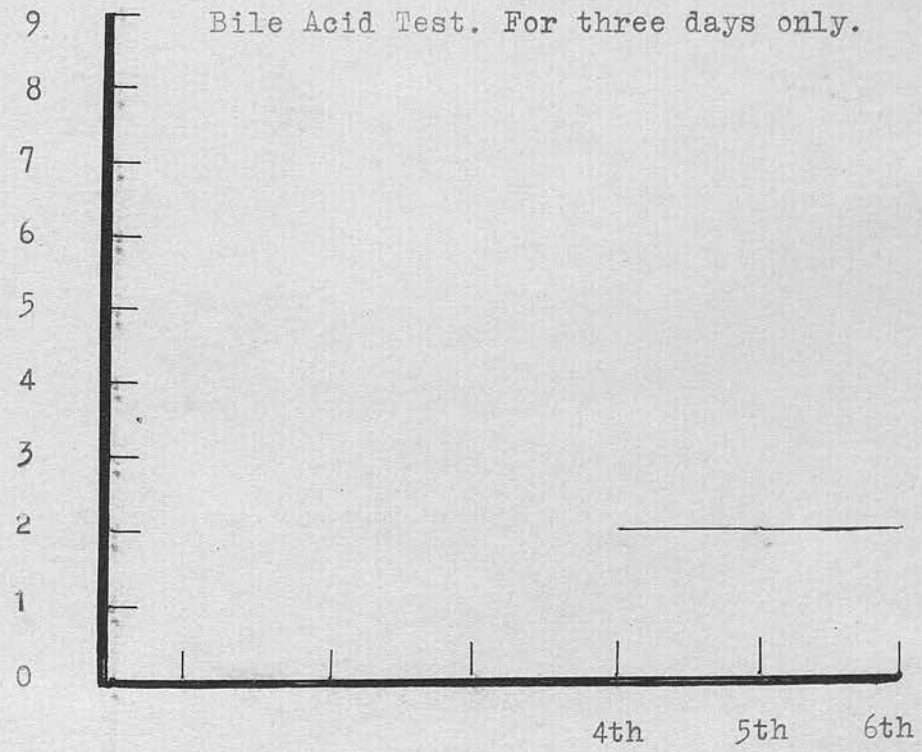


Another example of a urine moderately active

when examined over a period of six consecutive days.

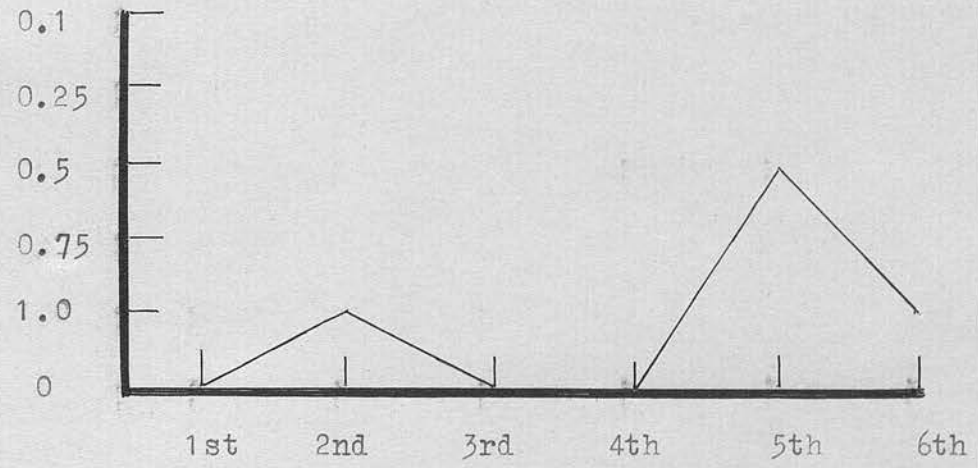
Case No. 26. J. F-. Female. Age 18.
Vomiting assoc. with
menstruation. Visceroptosis.
No drug treatment.



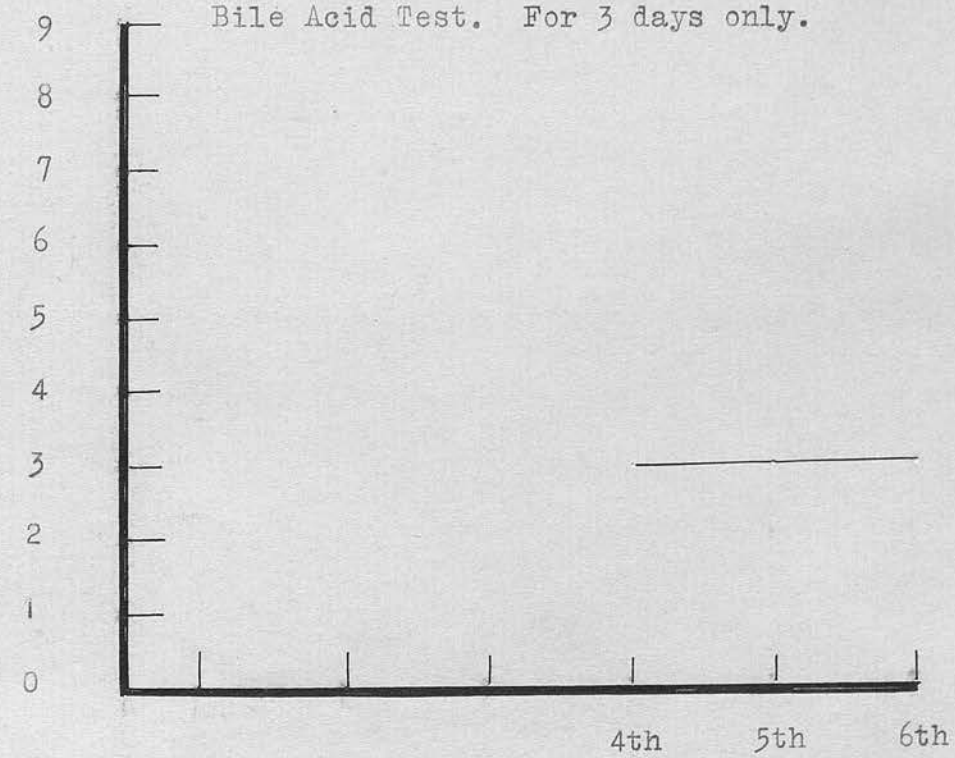


A moderately active specimen, on the whole. The dilution of urine at which complete haemolysis is produced varies greatly from day to day.

Case No. 27. A.McD. Female. Age 45.
Hyperchlorhydria.

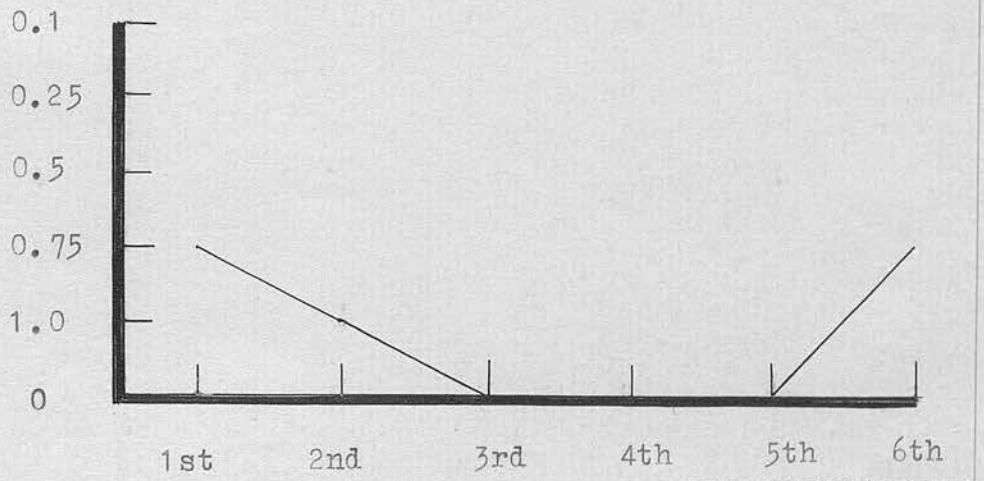


Bile Acid Test. For 3 days only.

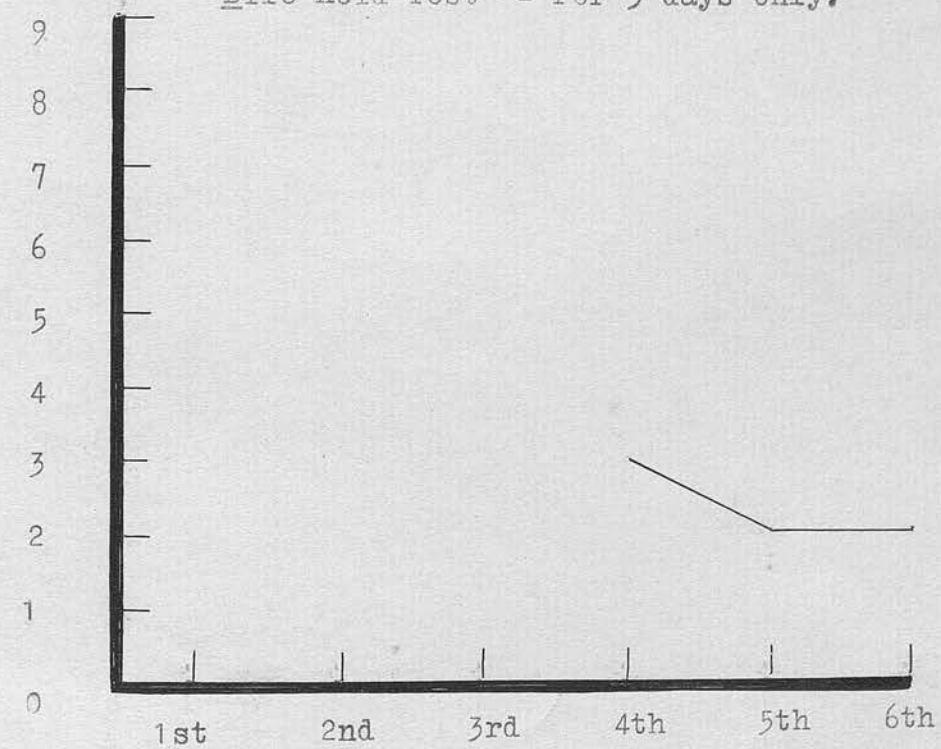


This urine again exhibits a non active period durin the time tested. Although the bile acid reading remains the same, the haemolytic activity varies.

Case No. 28. A.G-. Female Age.25.
Purpura Haemorrhagica.
Didymin gr V. t.i.d.



Bile Acid Test - For 3 days only.

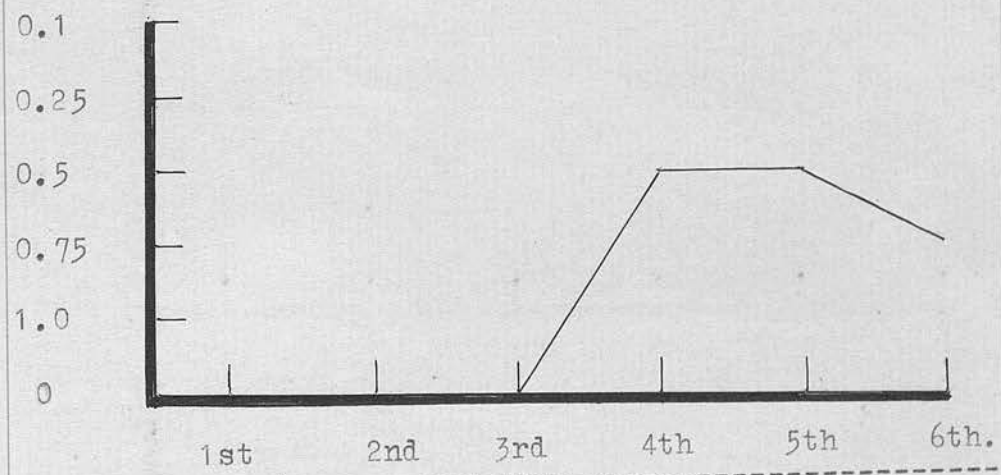


Another inactive urine, comparatively; no haemolysis occurs on three of the days tested, although there are no abnormal constituents, and the bile acid read -ing is higher on 4th day when no haemolysis occurs

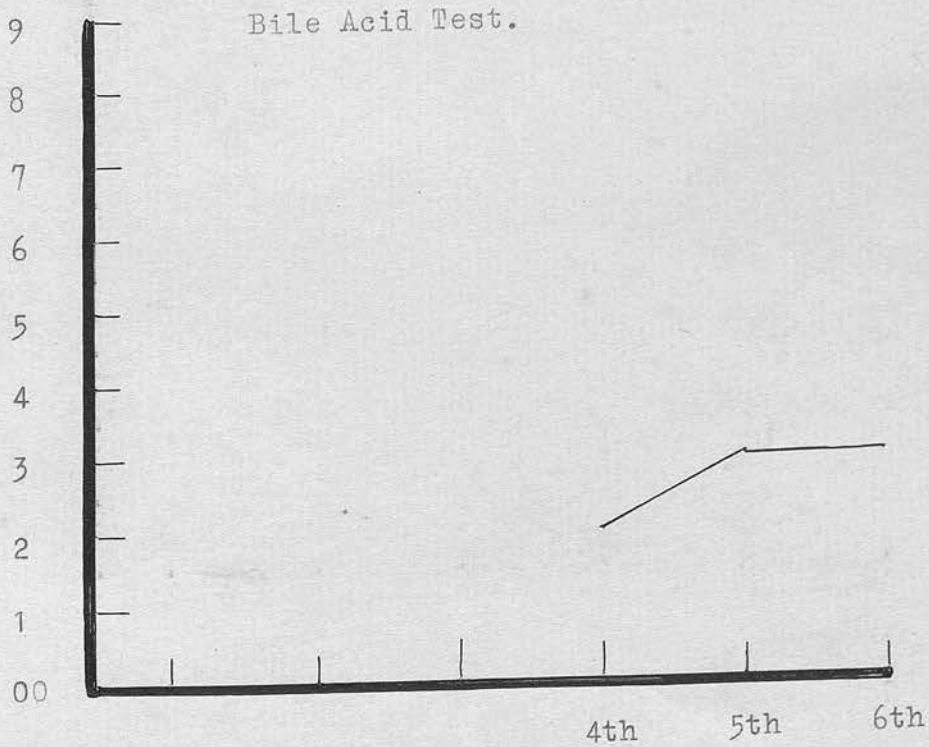
occurs/ than on the 6th when it occurs at 0.75.

Haemolytic Activity.

Case No. 29. I.M-. Female Age 27.
Exophthalmic Goitre.
Auricular fibrillation.
Tinct. Digitalis. m X. t.i.d.

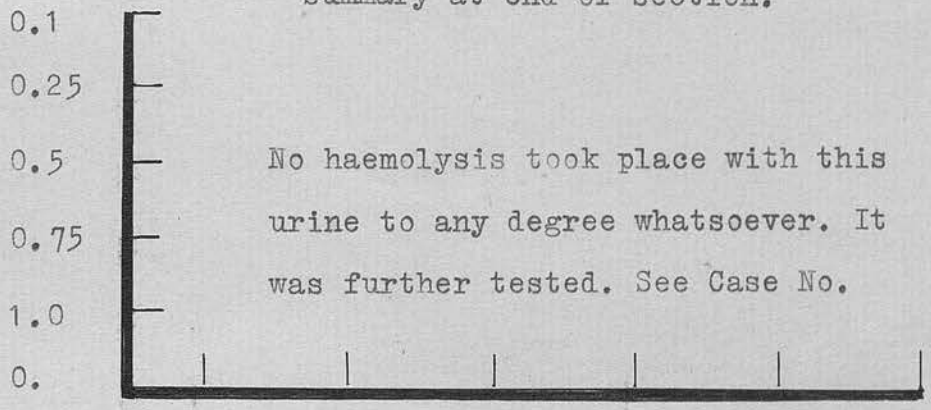


Bile Acid Test.



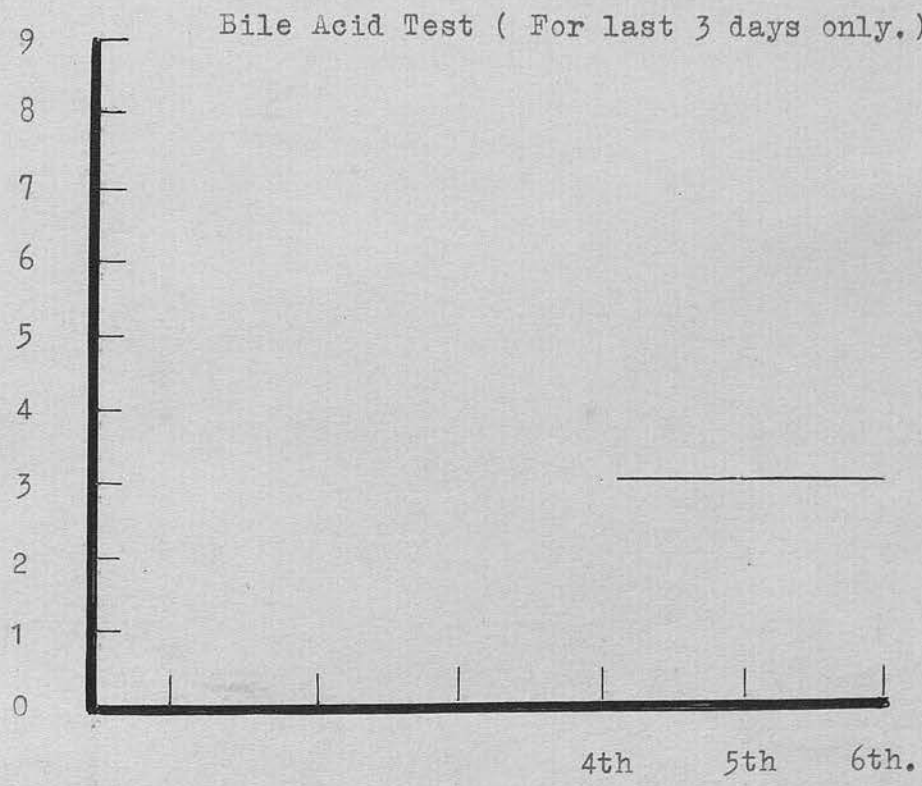
No haemolysis occurs for three days. This urine might readily have been described as non-haemolytic had it only been examined on these days.

Case No. 30. M-. Female . Age 34.
Pernicious Anaemia.
Enteretis - B. Pyocyaneus.
Urine - Culture of B. Pyocyaneus. p.m.
This case is mentioned in the summary at end of section.



No haemolysis took place with this urine to any degree whatsoever. It was further tested. See Case No.

Bile Acid Test (For last 3 days only.)



Moderate bile acid content comparatively, although the specimen will not produce haemolysis in any dilution or undiluted.

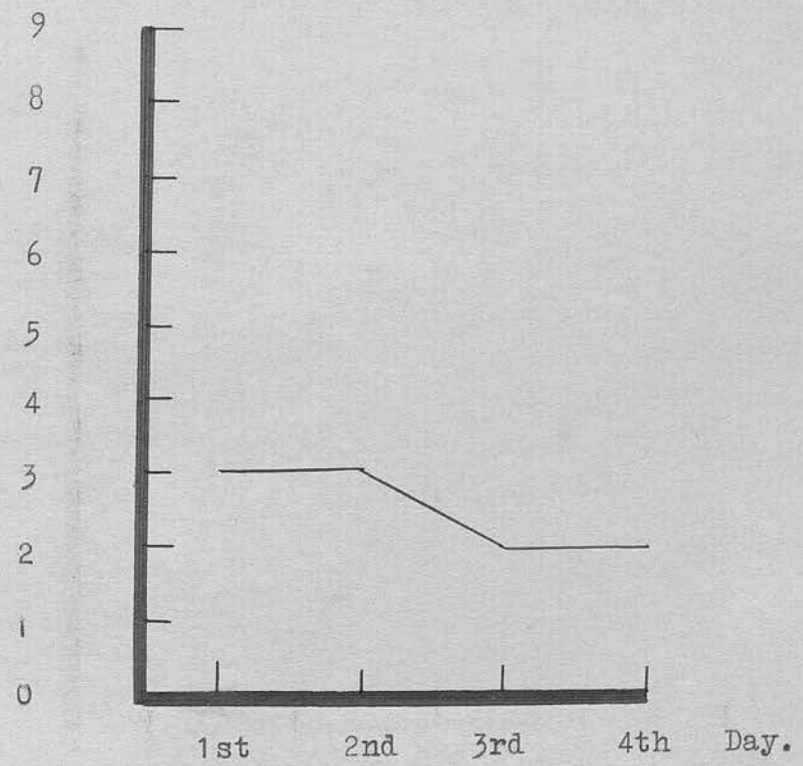
Case No. 31. A.R. Chronic Parenchymatous Nephritis.

Albumen content;- 1st day. 1.5 Grammes per Litre.
2nd " 1.4 " " "
3rd " 1.5 " " "
4th " 1.6 " " "

Blood present on 3rd & 4th days.

No Haemolysis took place on any of these days.

Bile Acid Test.
for these four days.

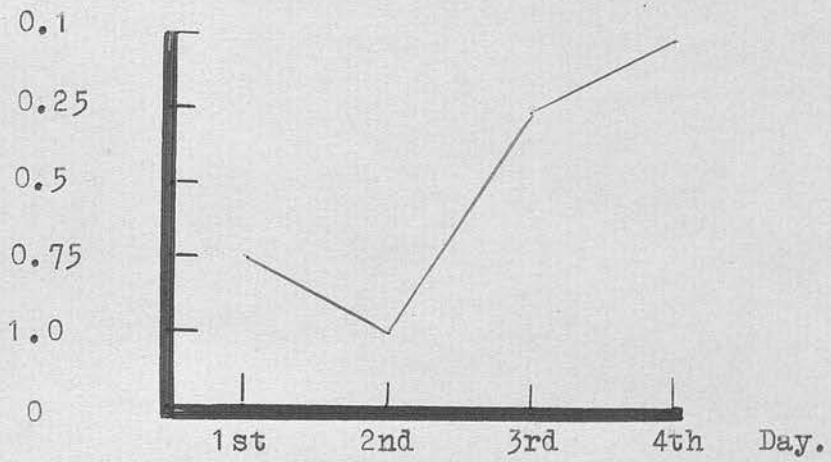


Another example of the prevention of the haemolytic action ; in this case due both to the presence in the urine of blood (serum) and albumen.

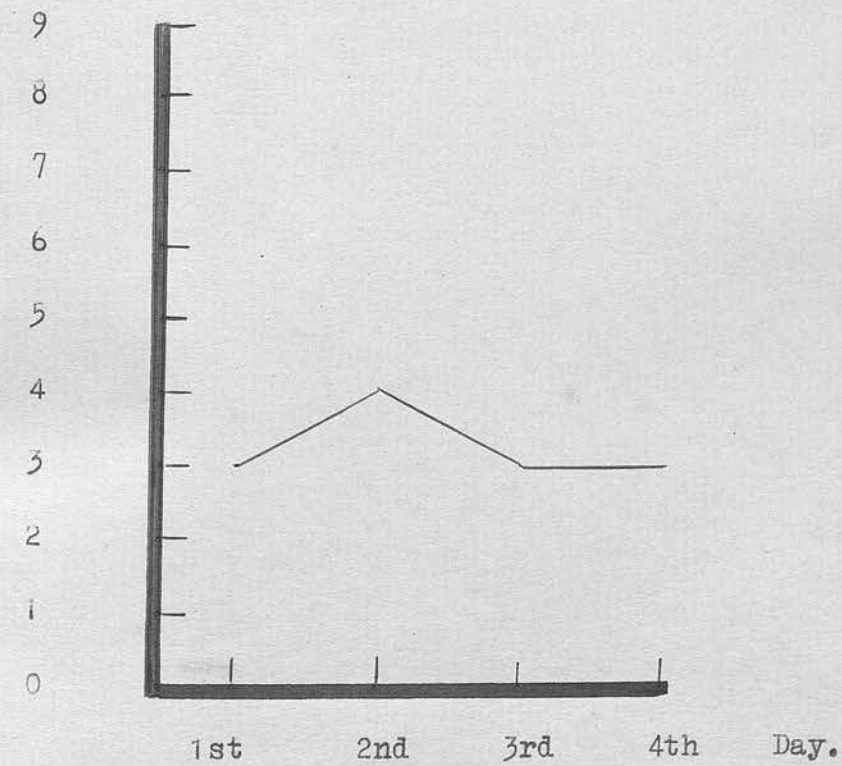
Haemolytic Activity.

Case No. 32

L.M. Female. Age 15.
Simple Goitre. (colloid.)
Ext. Thyreoid. gr.iij. t.i.d.

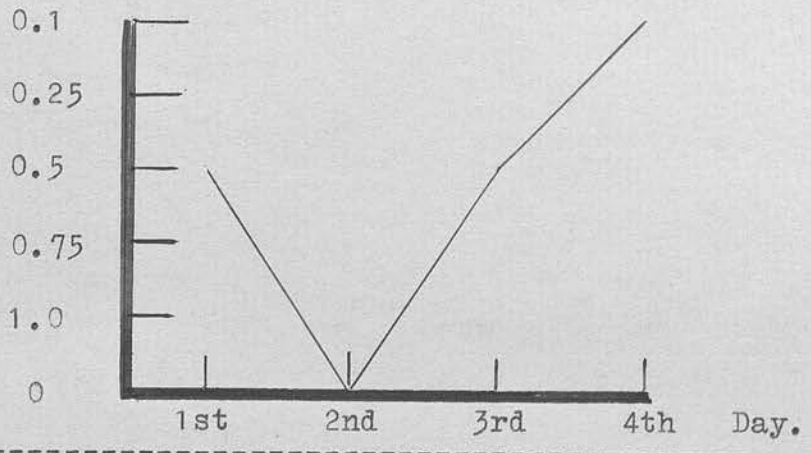


Bile Acid Test.

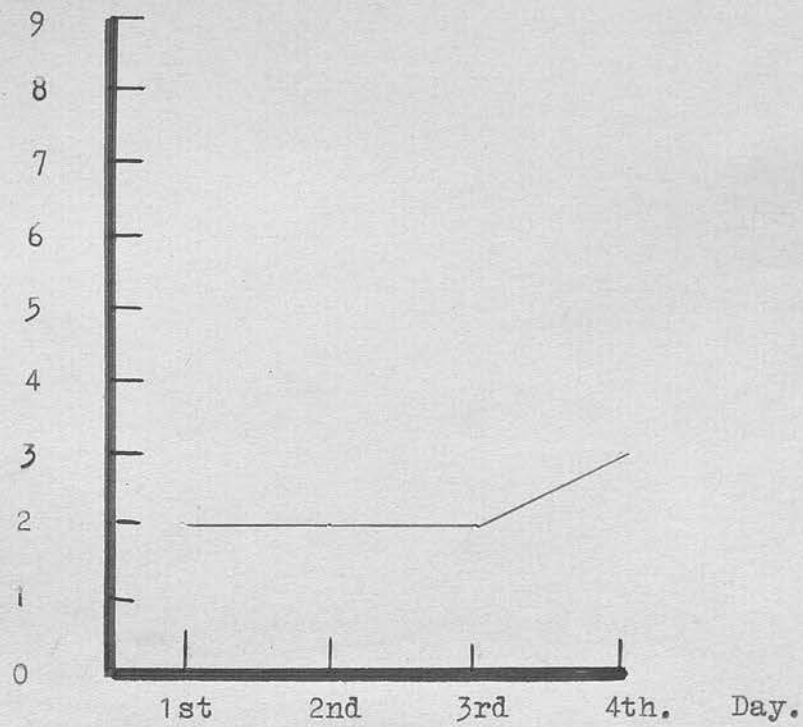


No parallelism exhibited in these two records.

Case No.33. E.M. Age 36. Female.
Duodenal Ulcer?
No drugs given during this period.



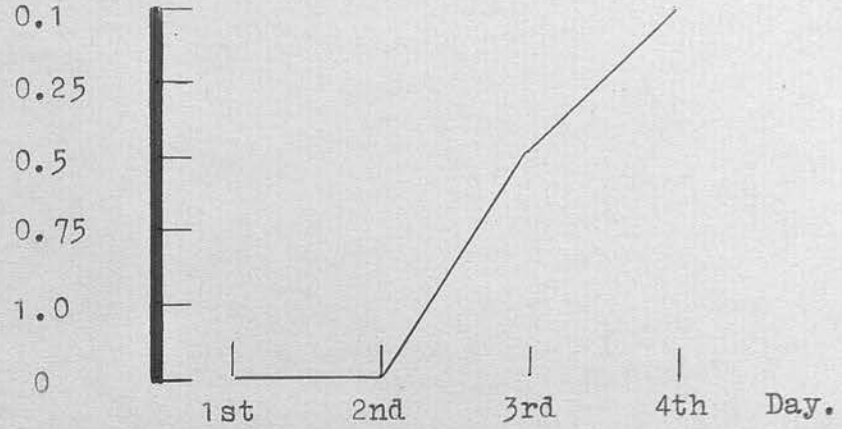
Bile Acid Test.



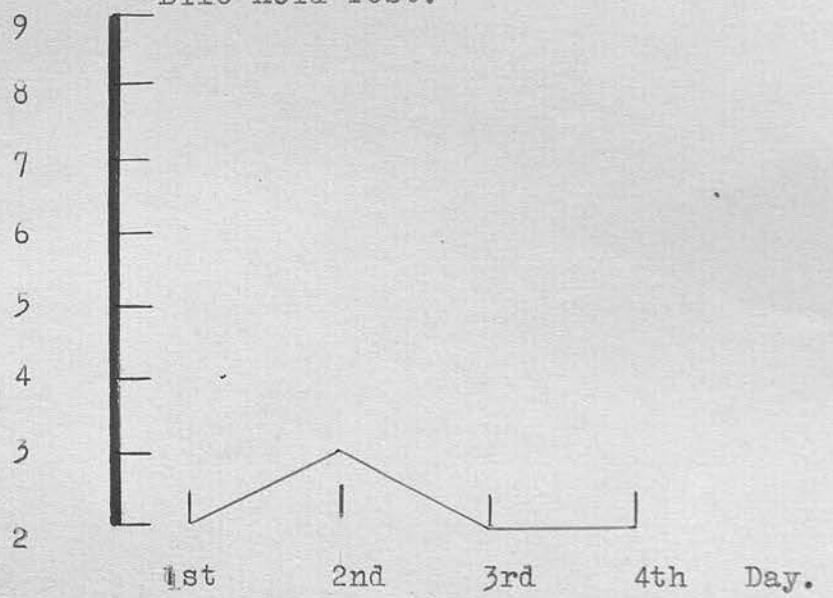
No haemolysis has taken place on the 2nd day. Even after a further hour's incubation (i.e. 5 hours') no haemolysis occurred. There is, however, no corresponding drop in the bile acids apparently.

Case No. 34.

Mrs.F. Age .?
Hyperthyreoidism.
No drugs given which could affect the haemolytic power.

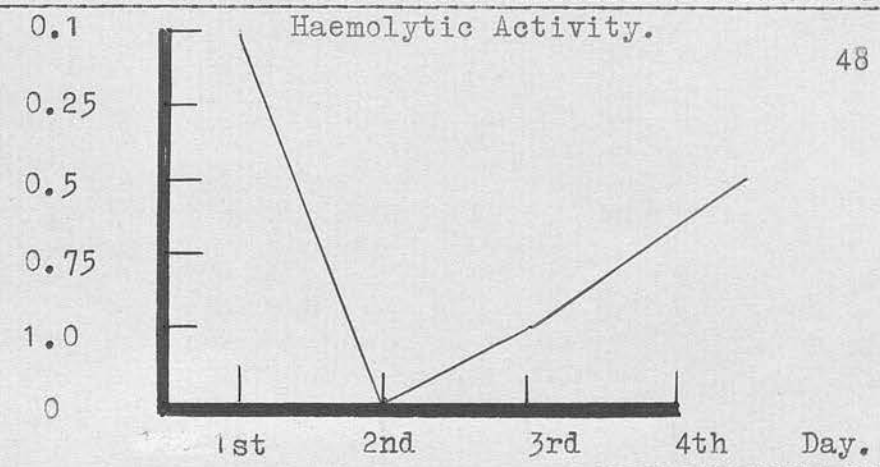


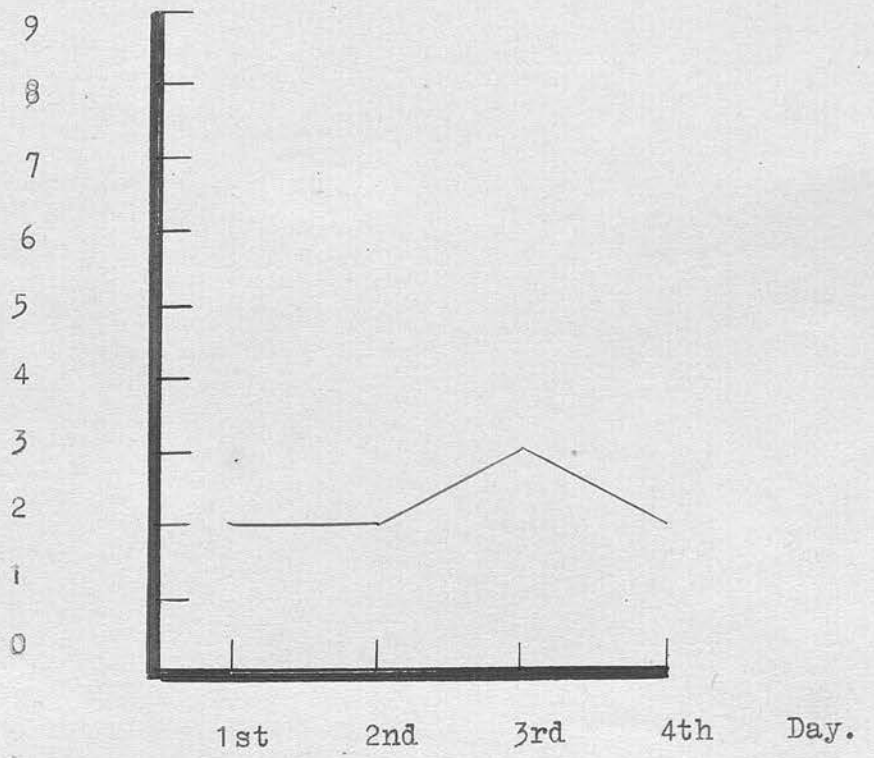
Bile Acid Test.



We do not find a parallelism between the two graphs in this instance. On 1st & 2nd days it will be noticed that no haemolysis occurs. This is, of course, with 4 hours incubation. It does not signify that in in all such cases the urine is absolutely non-haemolytic; e.g. on the 2nd day here haemolysis occurred at 0.5 c.c. in 5 hours. Others certainly may not produce haemolysis after many hours incubation and

and/ can be called non-haemolytic. Even if they do produce haemolysis after say, 12 or more hours incubation other factors must be considered, apart from the surface acting substance involved and described in a later section.





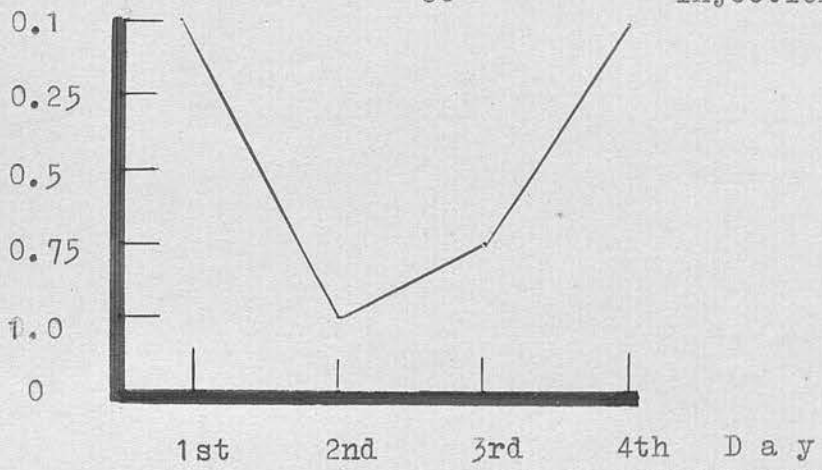
No similarity in the bile acid readings taken in conjunction with the activity of the haemolysin.

Case No.35. N.S-. Visceroptosis?

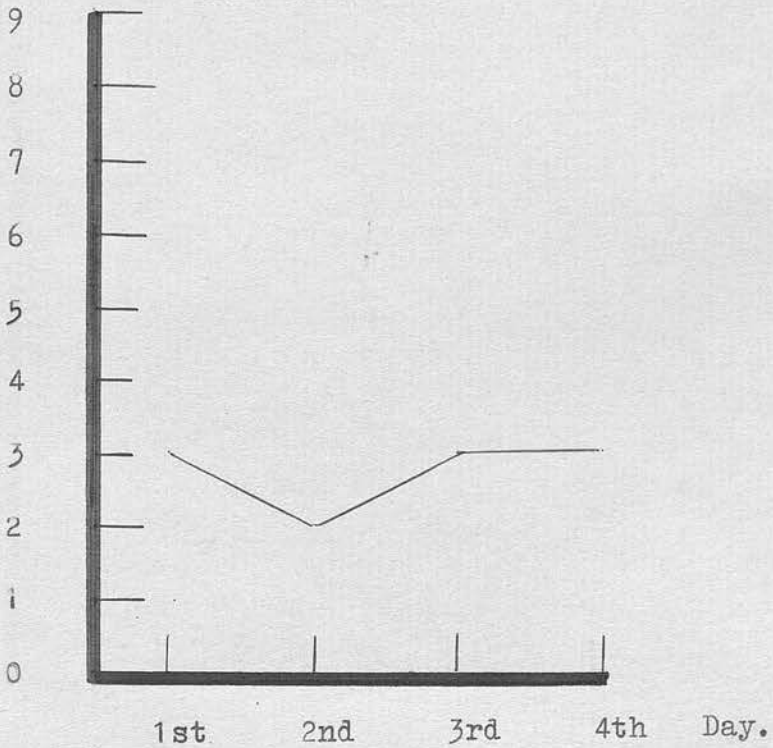
Sugar present on 1st, 2nd, 3rd. days. Very slight on 1st day.
Drugs= $\frac{1}{60}$ Strychnine 4 hrly. injection.

Case No. 36.

R.C. Female. Aged ?
Migraine.
Drugs given= $\frac{1}{60}$ Strychnine 4 hrly. injection.



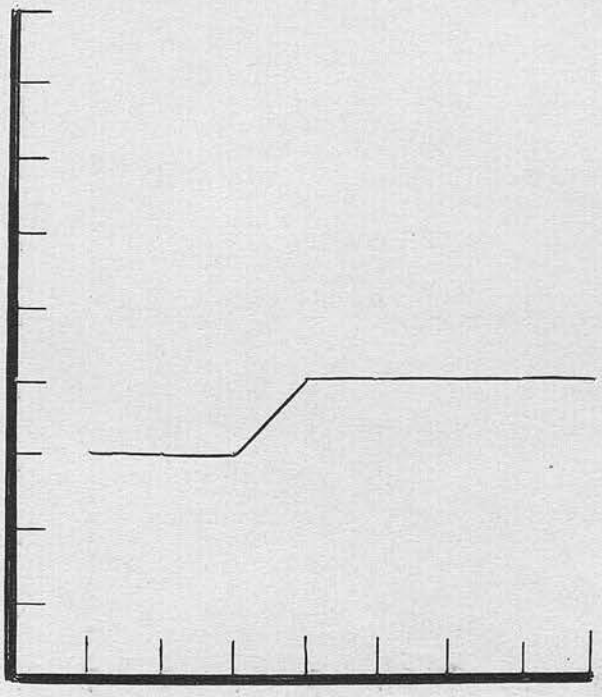
Bile Acid Test.



This certainly shows a slight correspondence. There is a drop in the bile acids on the 2nd day and a rise on the 3rd corresponding with the variation in haemolytic activity.

Case No. 37.

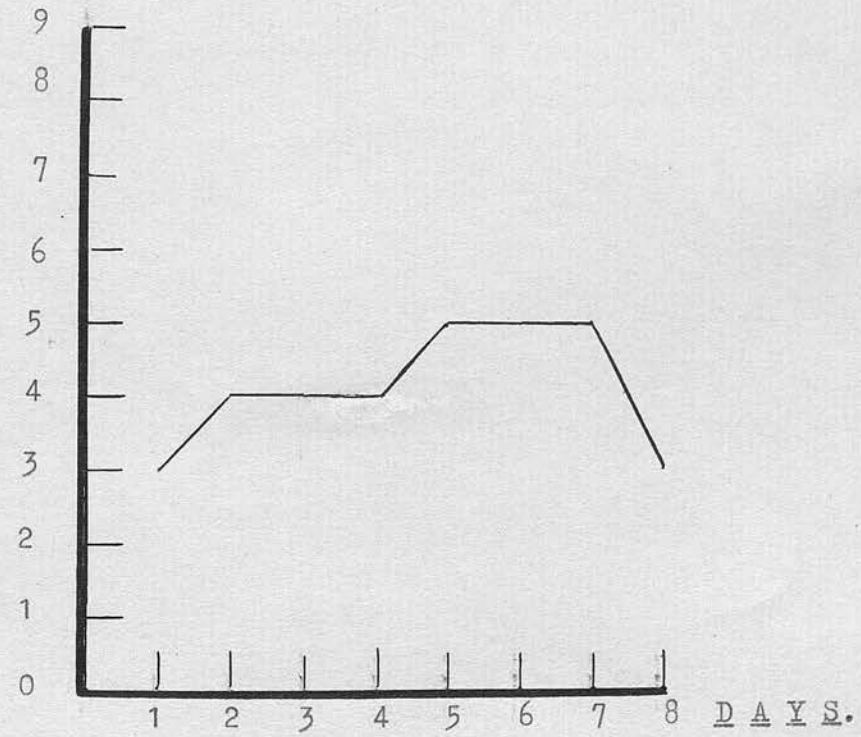
_ Being the urine of Case No. 30 also observed for a further period of five days as no haemolysis had taken place during the previous testing. In this case however no abnormal constituents e.g., albumen, pus, or blood, were present which would hinder or prevent the occurrence of haemolysis. Still no haemolysis was produced. The bile acid



content of the specimens, too, are relatively high, as seen from the above graph. These urines similar to the above are discussed in the summary.

Case No. 38.

This is the urine of Case No.19. observed for a further five days as there was no haemolysis produced when previously tested. No haemolysis graph is given because after this further per- still no haemolysis was produced. The reason is that the specimen contained albumen. A series of specimens containing substances which retard



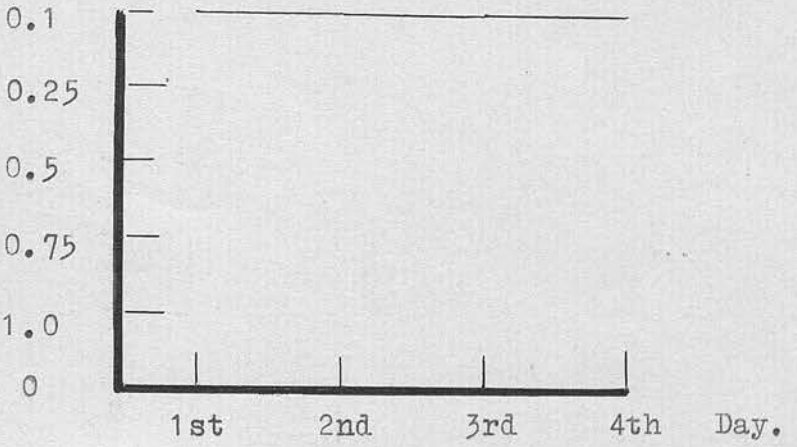
or prevent haemolysis is given elsewhere where these are discussed. It will be noticed here that the bile acid content taken over eight days is relatively high.

The third and last series of graphs are similar to the previous series, with the further addition of the readings of the specific gravity of the urines during the period of experiment, in order to ascertain if there was any parallelism between the specific gravity and the degree of haemolysis. The specific gravity was read by means of the ordinary urinometer. A newer instrument, which is reputed to be more accurate and one which is said to be more convenient in that it can be used with smaller quantities of urine, was tried and discarded owing to many disadvantages found during its use.

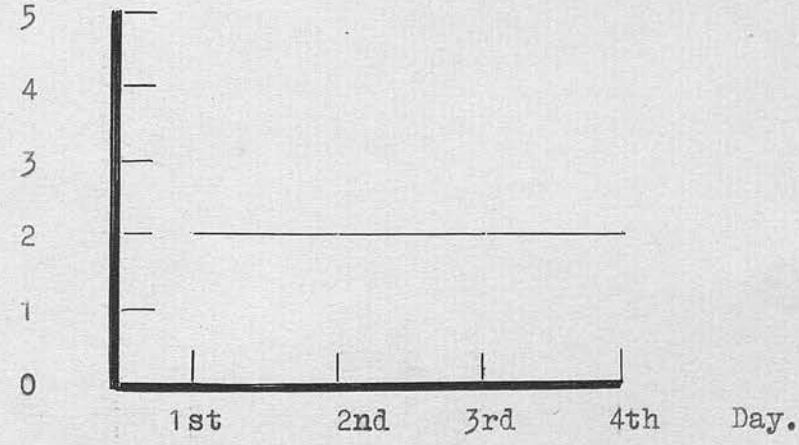
The controls (1.0 c.c. 0.85 % NaCl, with 1.0 c.c. blood suspension incubated for a corresponding time at the same temperature.) were all negative. No haemolysis taking place to any degree in any of these.

Haemolytic Activity.

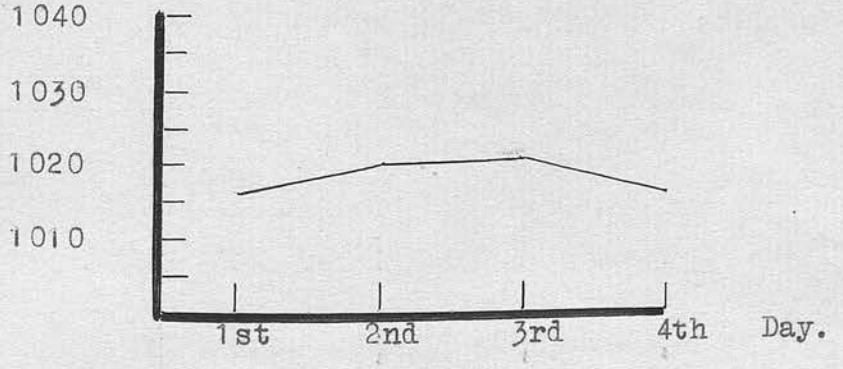
Case No 39.. B-. Age 46.
Chronic Bronchitis. History of Pulmonary Tuberculosis.



Bile Acid Test.



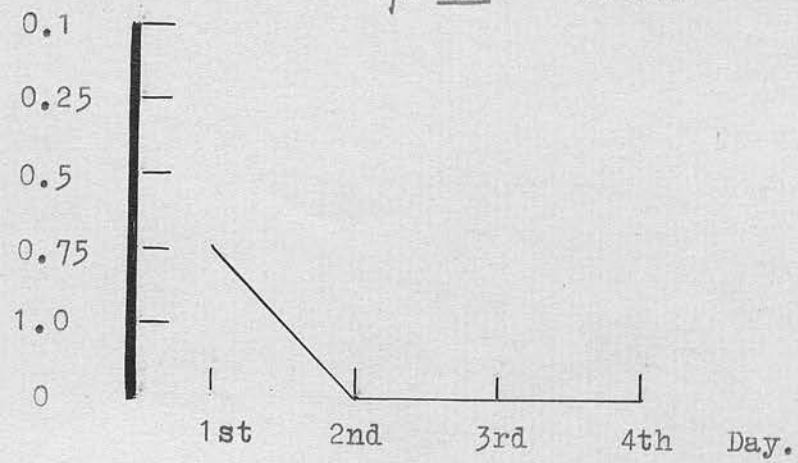
Specific Gravity.



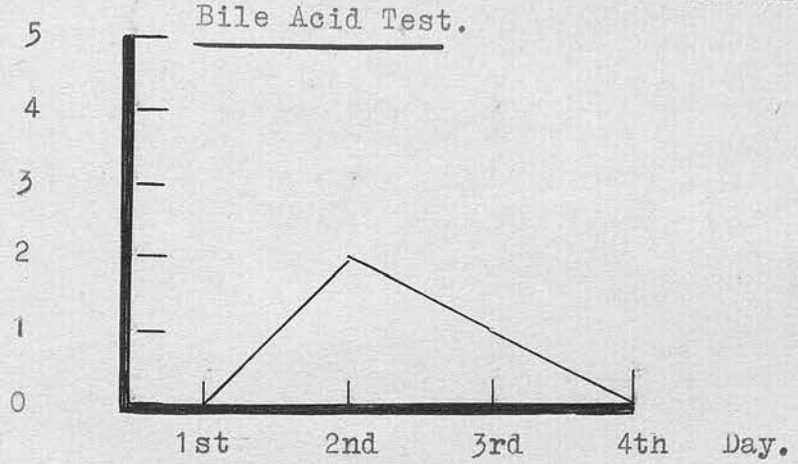
A very active specimen. At first glance the bile acid readings would appear to correspond entirely with the haemolytic activity but the bile acid content it will be noted is relatively very small during all the days the urine has been examined.

Haemolytic Activity.

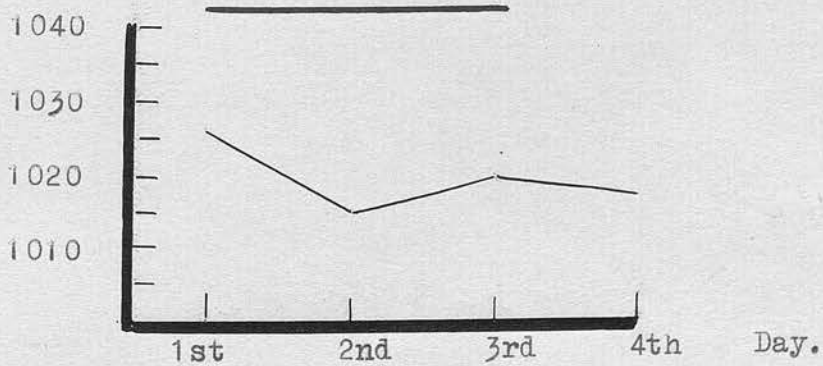
Case No. 40. N-. Age 48.
Pulmonary Tuberculosis.
Patient got Sodii Bicarbonatis
gr. XX — t.i.d.



Bile Acid Test.



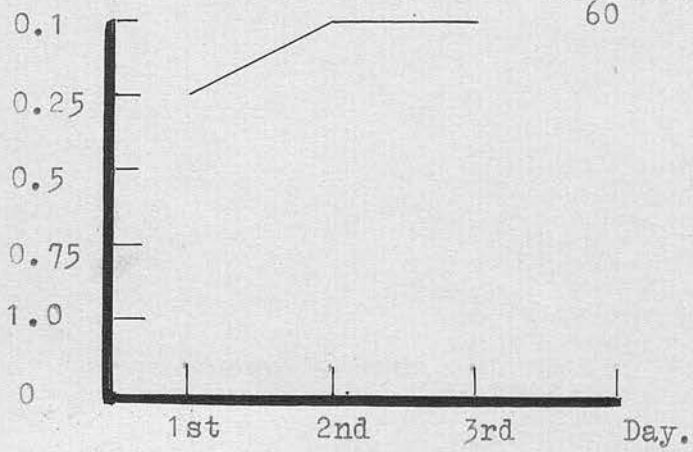
Specific Gravity.



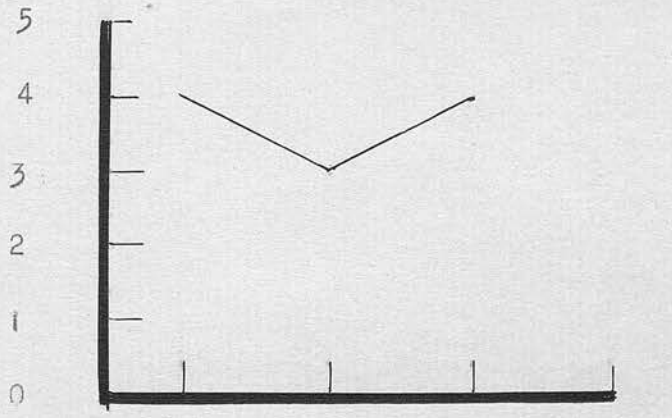
A very inactive specimen, but the urine contained sugar (trace) on 2nd day. Moreover the patient had Sod.Bicarb. daily which has been found to inhibit the haemolytic action and may account for the inactivity during the last 3 days.

Haemolytic Activity.

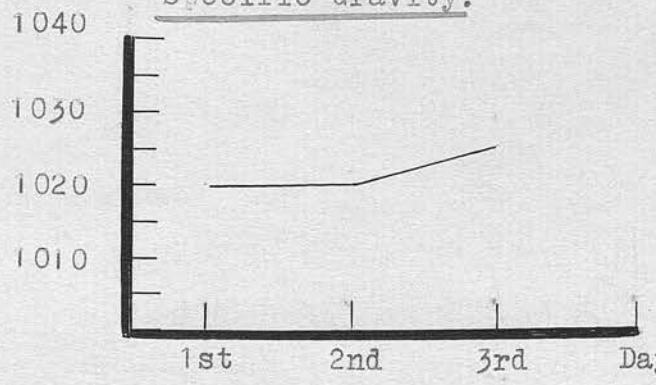
Case No. 41 . K-. Female .Age 22.
Pulmonary Tuberculosis.
Drugs= Strychnine gr $\frac{1}{60}$ daily.



Bile Acid Test



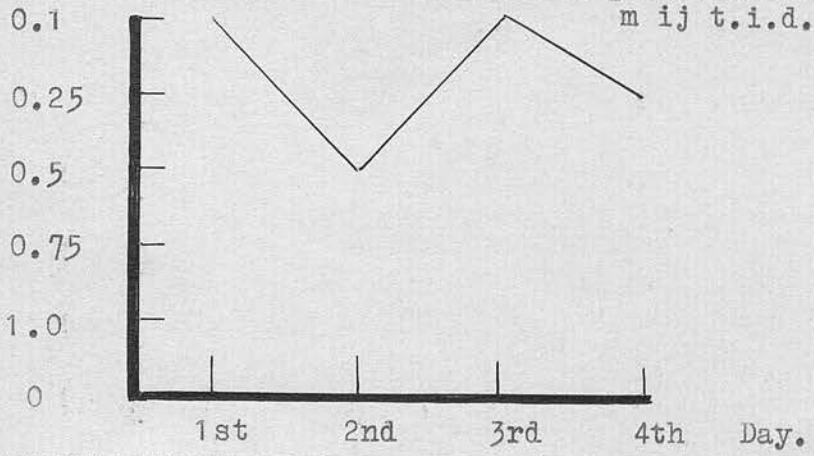
Specific Gravity.



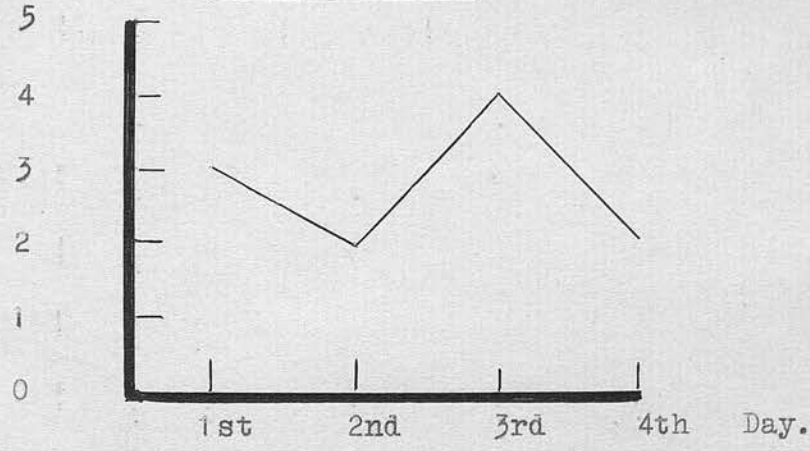
A comparatively high bile acid reading and a comparatively active urine. There is no drop in the haemolytic activity,however, on the 2nd day agreeing with the fall in bile acids.

Haemolytic Activity.

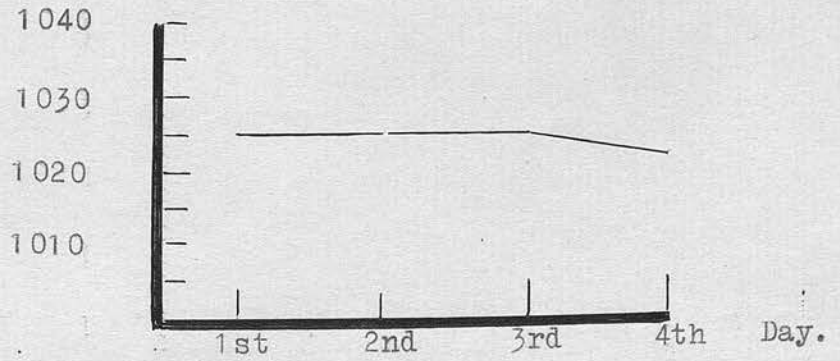
Case No. B-. Male Age 12. (42)
Pulmonary Tuberculosis.
Drugs in Treatment = Liq Arsenicalis m ij t.i.d.



Bile Acid Test.



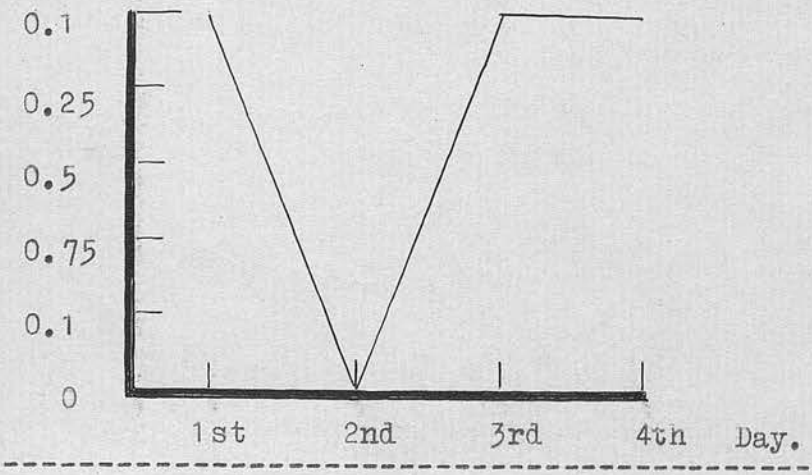
Specific Gravity.



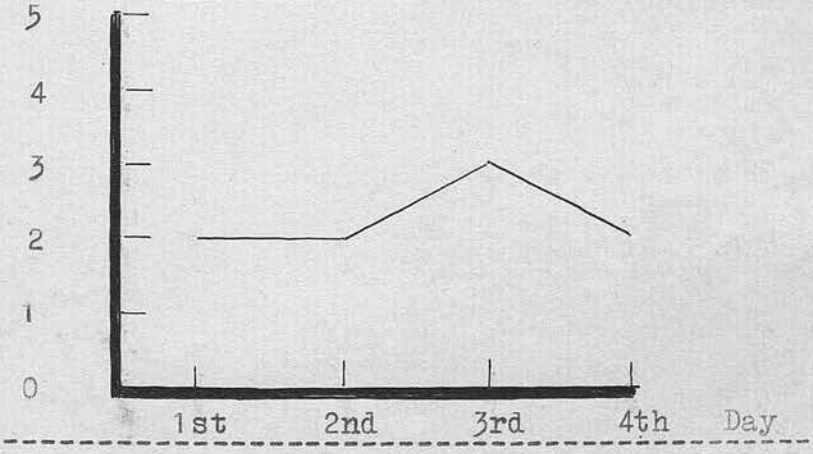
Here is one of the few examples from these experiments where the bile acid content runs parallel to the haemolytic power of the urine. There is no variation in the specific gravity for 3 days although the activity of the urine varies.

Haemolytic Activity.

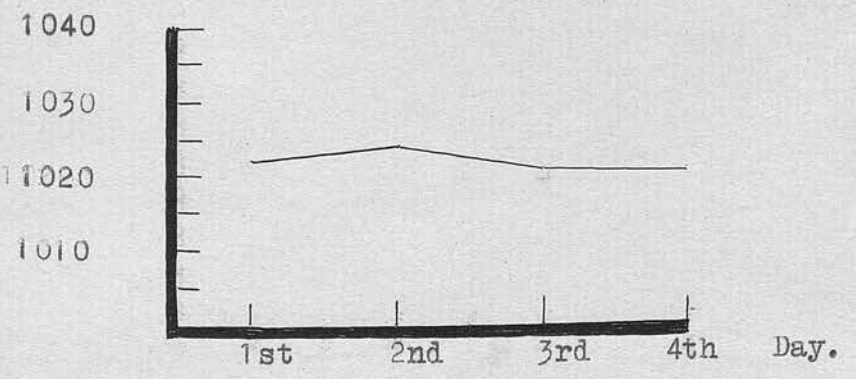
Case No. 43. L-. Age 23. Male.
Chronic Bronchitis.



Bile Acid Test.



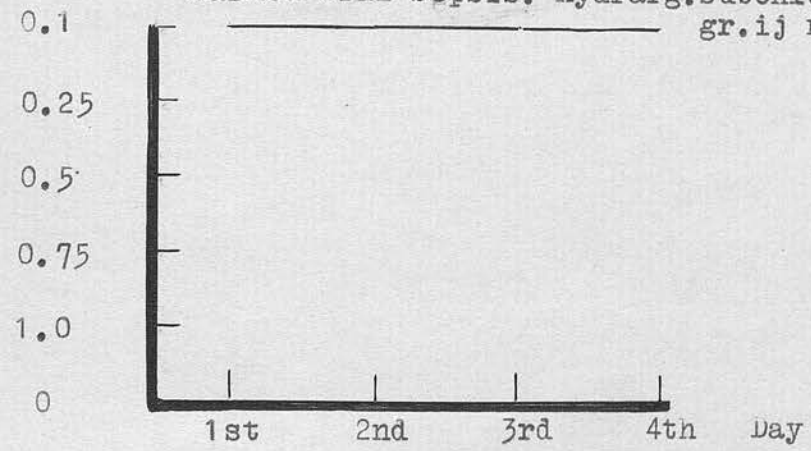
Specific Gravity.



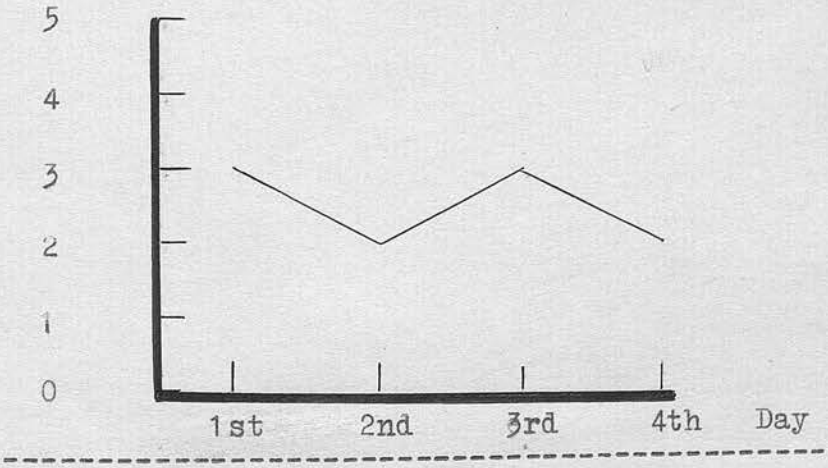
There is no fall in the bile acids corresponding to the loss of haemolytic power of the urine on the 2nd day. Similarly there is no agreement in the specific gravity readings.

Haemolytic Activity.

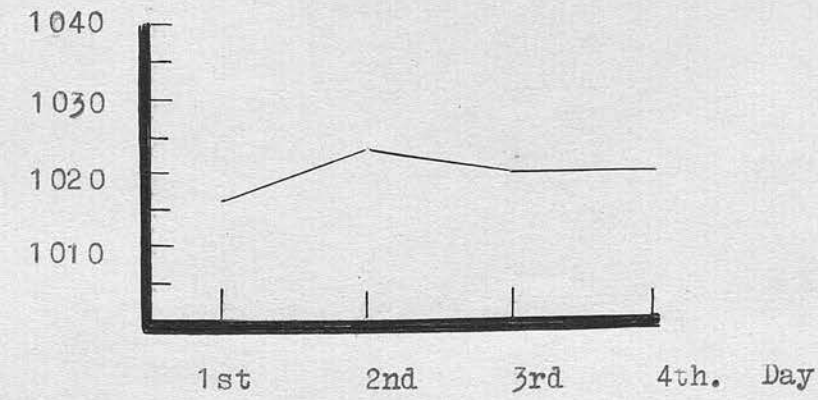
Case No. 44. B-. Age 19. Male.
Pulmonary Tuberculosis.
Marked Oral Sepsis. Hydrarg.Subchlor gr.ij nocte.



Bile Acid Test.



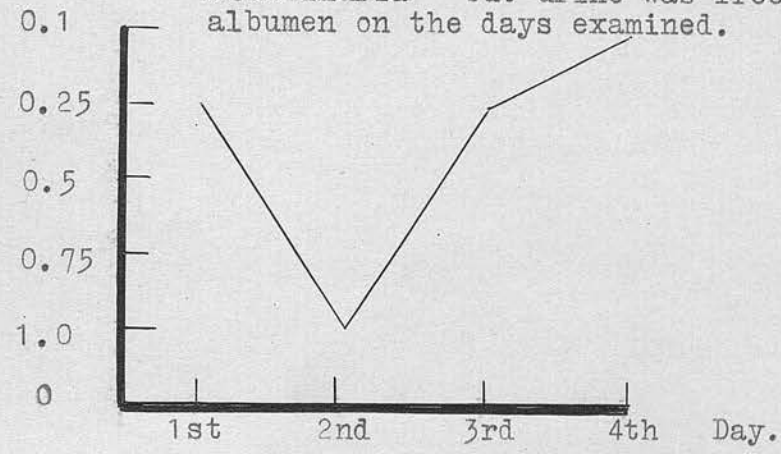
Specific Gravity.



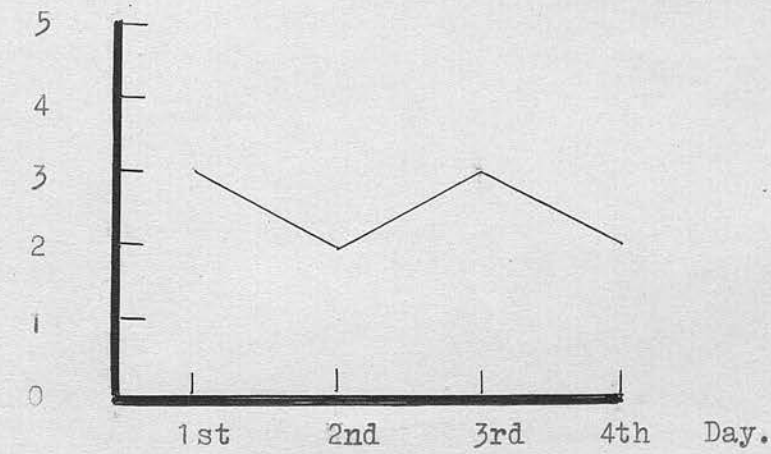
Another very active urine . The bile acids however do not agree. Neither does the specific gravity appear to be related to its activity.

Haemolytic Activity.

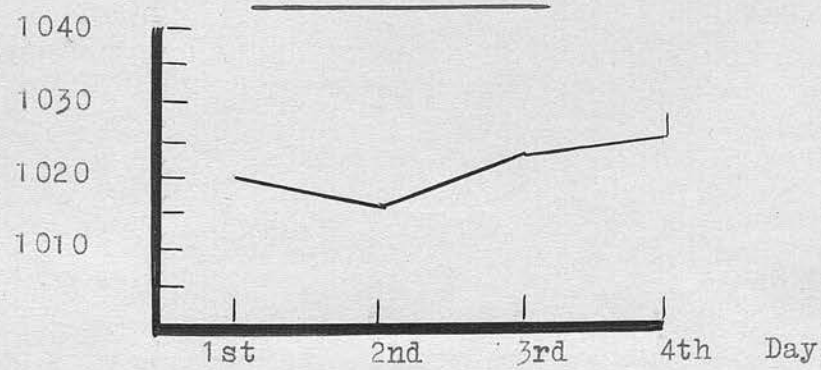
45. Case No. D-. Age 26. Female.
Pulmonary Tuberculosis.
Albuminuria - but urine was free from albumen on the days examined.



Bile Acid Test.



Specific Gravity.

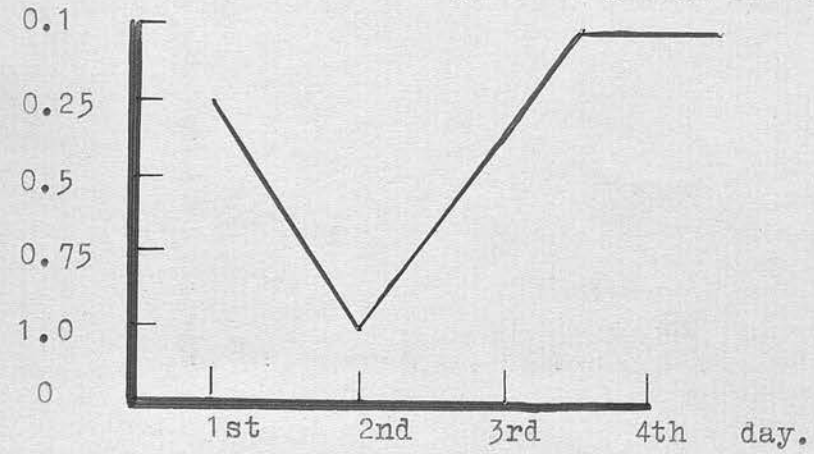


For the first 3 days the bile acid content runs parallel to the haemolytic activity of the urine. In this case the specific gravity appears to bear some relationship also.

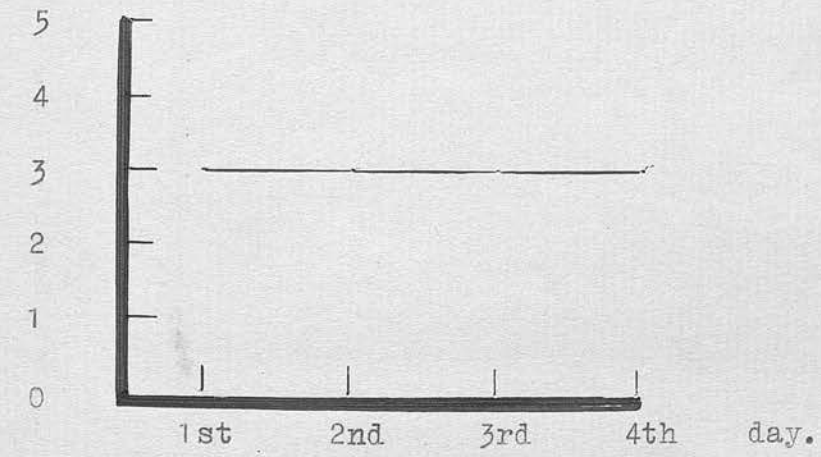
Haemolytic Activity.

Case No. 46. McA-.

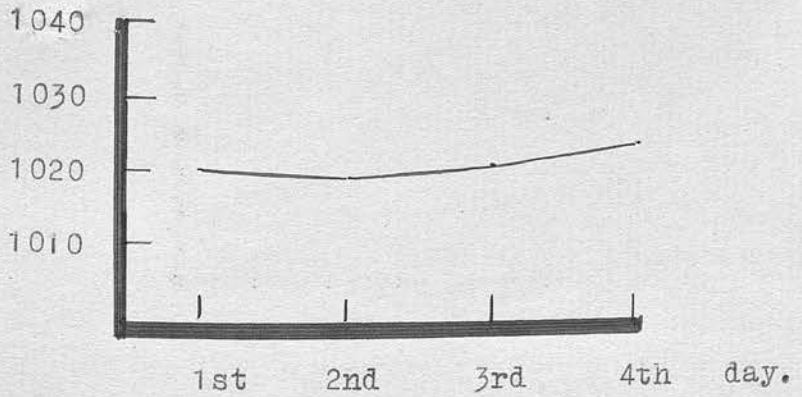
Pulmonary Tuberculosis.
No drugs during this period.



Bile Acid Test.



Specific Gravity.

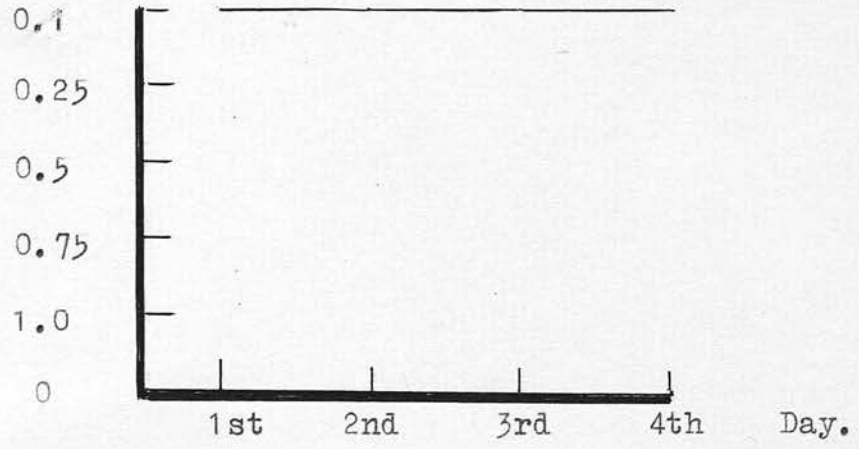


No Relationship between these three graphs.

Haemolytic Activity.

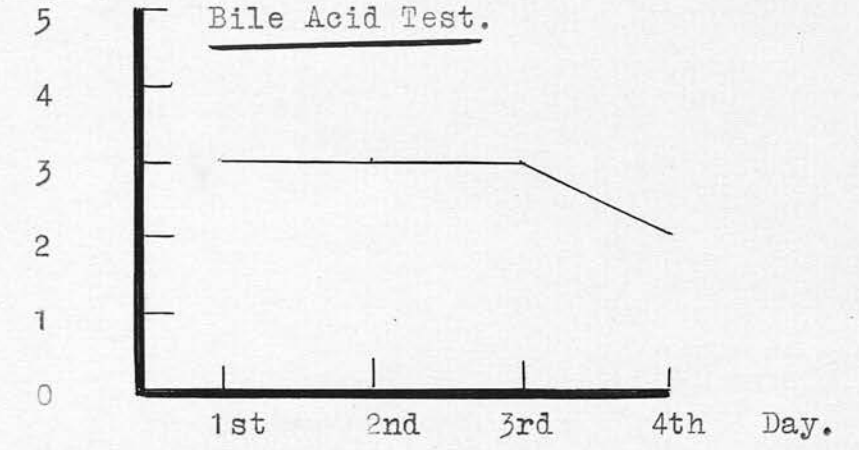
Case No. 47 M. Male. Age, 16

Pulmonary Tuberculosis. No drug treatment.

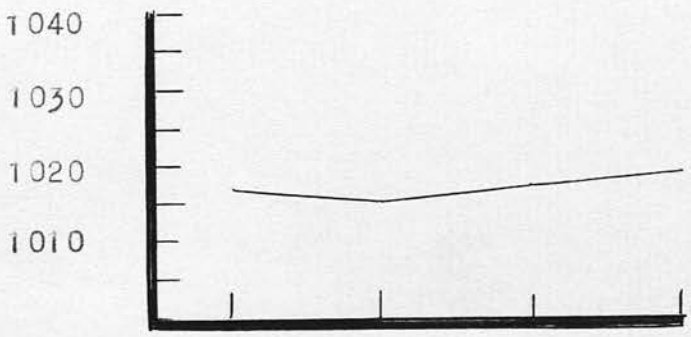


A very active urine

Bile Acid Test.



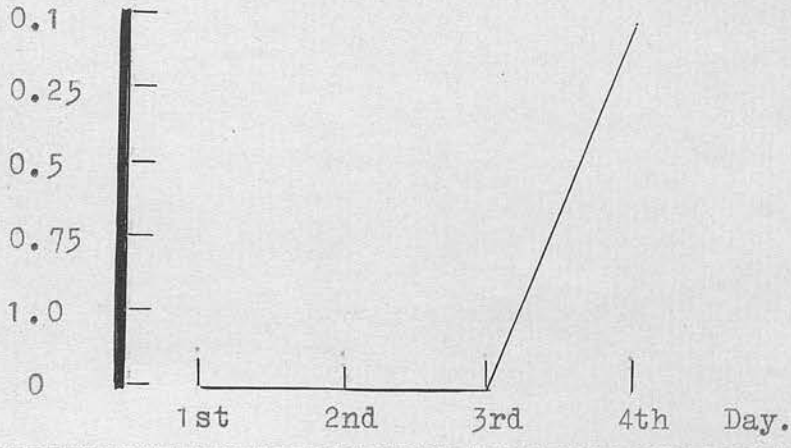
Specific Gravity.



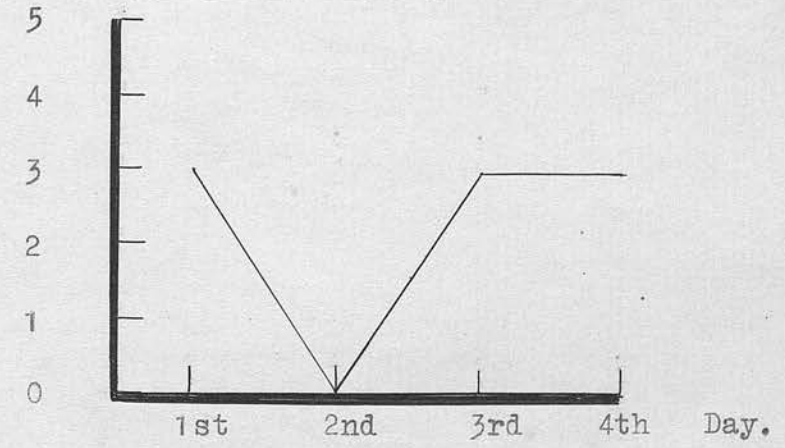
The bile acids are moderate in amount, but fall on the 4th day while the haemolytic remains the same. Practically no variation in specific gravity.

Haemolytic Activity

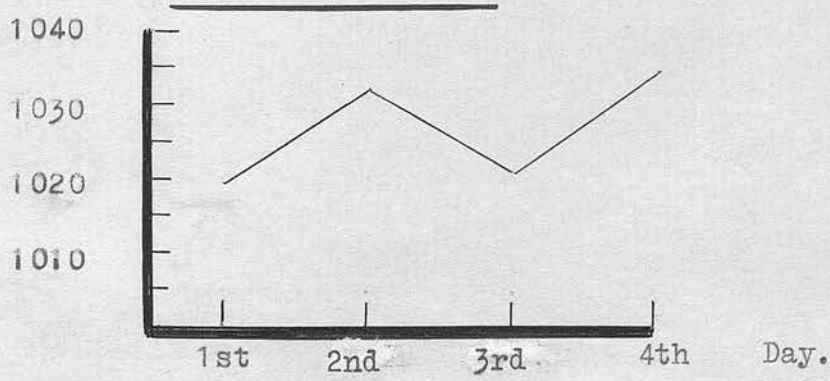
Case No.48.W-. Female aged 19.
Pulmonary Tuberculosis.
Artificial Pneumothorax.
Drug Treatment during this period
= Liq.Arsenicalis mij t.i.d.



Bile Acid Test.



Specific Gravity.

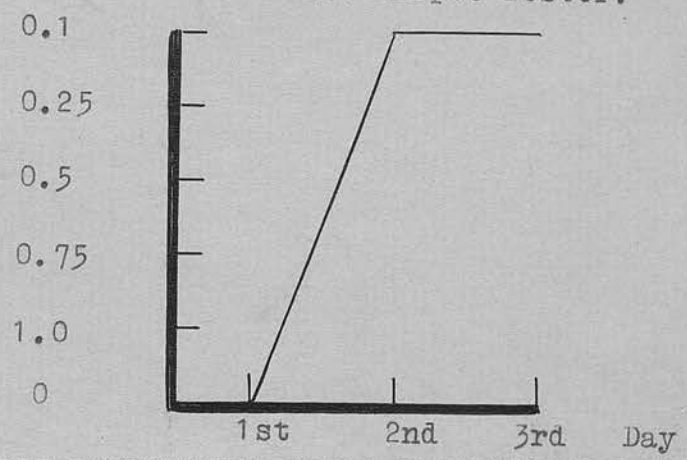


Urine haemolytically inactive for 3 days then very active on the 4th day. Although there were apparently no bile acids present on the 2nd day, the readings for the 1st and 3rd days are moderately high.

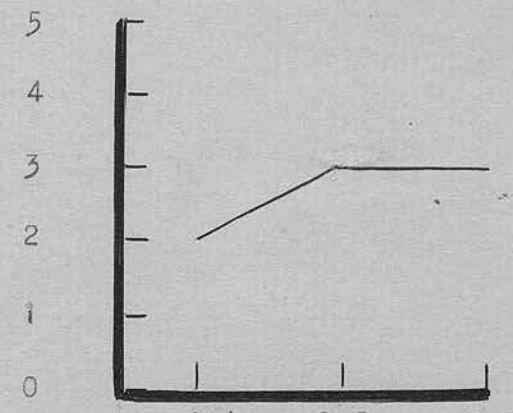
The specimen contained sugar by Fehling's Test on the 2nd and 4th days. Neither the bile acids or the specific gravity appear to have any bearing on the haemolytic activity.

Haemolytic Activity.

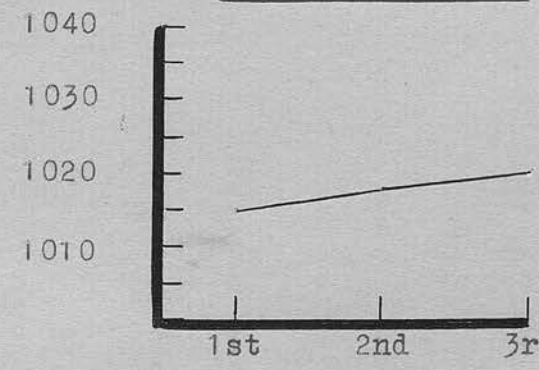
Case No.49.. D- Age 18. Male. Pulmonary Tuberculos -is. Herpes Zoster.



Bile Acid Test.



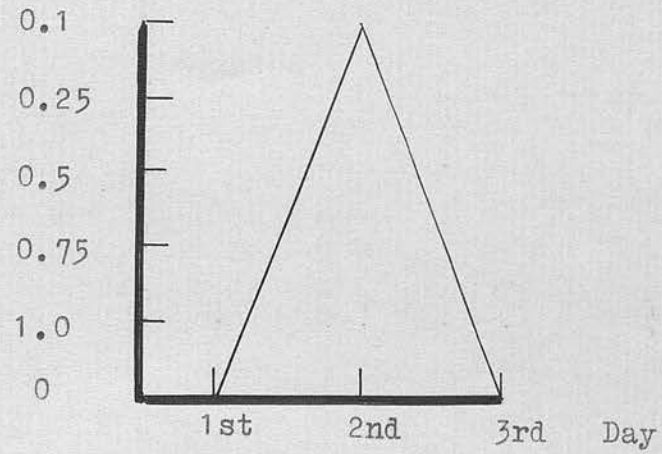
Specific Gravity.



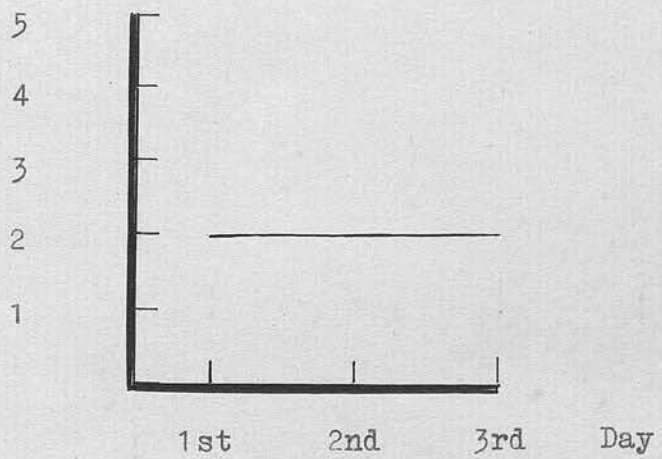
Although there is no activity on the first day, many otherurines have been examined where the phenomenon has been absent although there have been no abnormal constituents. Nevertheless this patient occasionally had a trace of albumen,and while none was apparent on these days the fact is worth mention as the specimen is very active on the last two days.

Haemolytic Activity

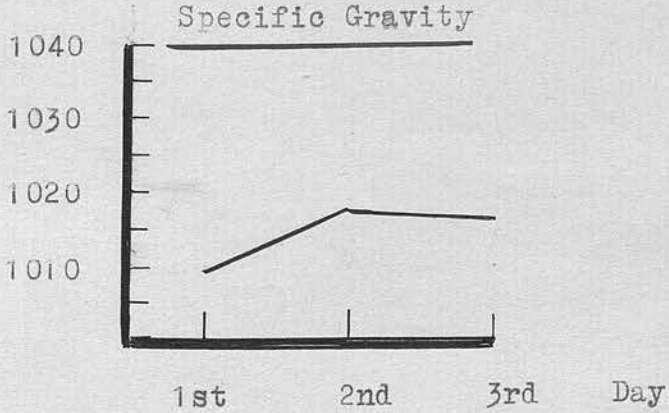
Case No. 50. C-. Age 48 Male
Cystitis. Pulmonary Tuberculosis.
Drugs.= Ac. Sod. Phos. gr. XXX
Hexamine gr. X
Urine observed for 3 days only.



Bile Acid Test



Specific Gravity



While the haemolytic power of this spesimen is seen to vary the bile acids remain constant inaamount. It is possible that there was a small amount of pus present but it was not examined.

SUMMARY.

McKee in his attempt to find some reaction whereby the conditions of so-called primary and secondary anaemias could be more sharply separated one from the other, thought it worth while to try to discover whether changes in the blood plasma led to changes in the blood corpuscles, and to find whether these changes, if any, were due to the loss of some protective material; to the presence of toxins, or to the presence of some substance passed into the plasma for the purpose of neutralising certain toxic elements - a something which while neutralising these toxic bodies exerted a coincident deleterious action on the erythrocytes. He thought it deserving of investigation to ascertain if bodies of the above type, if they occurred, were excreted, and if they were detectable in the urine. His first investigations on the haemolytic power of urine, therefore, were on the urines obtained from patients suffering from various types of anaemia, and he accordingly sought to find if a difference was apparent between the haemolytic activity of urine of healthy individuals and the urine obtained from cases of pernicicus anaemia.

It is curious that McKee never obtained haemolysis to any degree in the blood suspension he used - (the/

(the same strength as used by Ponder and as used in these experiments), - with the urine of normal individuals. One might ascribe this to the fact that he only incubated these normal urines for 2½ hours while the haemolytic phenomenon might easily, as has been fully shown in a later section, not have been apparent within this time; these urines having been classed as non-haemolytic while they were in reality haemolytic perhaps in most cases, although not in this period of incubation. Nevertheless his observation would indicate that, even with a longer period of incubation, the urine from certain pathological conditions would be much more haemolytic, obviously, if the phenomenon was apparent in 2½ hours in the specimens obtained from the morbid states he mentions, and absent with his normal urines. This fact has certainly not been borne out here. Fifty specimens of urine from healthy individuals were examined on one day and the results obtained, although contrary to McKee, are entirely in agreement with the results obtained by Ponder, who found 64% of normal urines to be haemolytic when examined on one day only. The figure obtained here was 58%. The amount of these 50 specimens (of urine just passed) taken, was 0.5 cc. and to each 0.5 cc., of 0.85% NaCl and 1.0 cc. of 0.5% human blood suspension was added. These were incubated for 4 hours at 42°C. (In controls of/

of 1.0 cc., 0.85% NaCl with 1.0 cc. suspension no haemolysis took place). Considering the urines from the unselected pathological conditions which were examined, we find the percentage taken daily over four of the days when these were examined, was as follows:- (i.e. only taking specimens which in 4 hours at 42°C. produced complete haemolysis at the 0.5 cc. dilution with saline and higher dilutions - e.g. 0.25 cc., 0.1 cc. - in order to agree with the dilution of the normal urines examined).

1st day	52%
2nd "	56%
3rd "	50%
4th "	54%

- a little lower even than the normal, but when one remembers that a few of the urines from the various pathological conditions contained substances which retard or prevent haemolysis; (e.g. albumen, pus, blood (serum)) and also that when haemolysis occurred at lower dilutions than that at which the readings of the normal were taken, e.g. 1.0 cc. (undiluted with saline) and 0.75 cc. these readings were omitted, - It will be seen that the percentage will at least be as high as the normal, if not higher - not so much higher however as to arrest attention.

Discussing urines of patients with pernicious anaemia, McKee states, that these exhibit this haemolytic action, and that just as the disease has periods of/

of remission so also does the haemolytic power vary. He does not however call attention to this observation as having any diagnostic or prognostic significance because he goes on to say that he observed the reaction present in a considerable number of other diseases which need not be enumerated here but will be found in his paper.

It is sufficient to say that in the urines of 50 unselected cases observed over a period of days, here, the reaction was present in all, on one day at least; and the variation in the haemolytic activity from day to day is well brought out in the experiments.- That is, the haemolytic activity was apparent in all cases - providing there was no abnormal constituent present in the specimen, such as albumen, pus, or blood, which will be shown later to prevent the haemolysis taking place, or at least retard the reaction.

It cannot be said that the urine of any of these pathological conditions was more actively haemolytic or less haemolytic than that of another, when observed over a period of days. It was thought at first that the urines of patients suffering from active pulmonary tubercular lesions were more actively haemolytic but in the 12 cases examined over 4 days this observation was not confirmed, when compared with the others. The urines given later, which were used for surface tension estimation in conjunction with haemolytic activity, were also urines from pathological conditions but/

but no note is given as to the disease, etc. as it was found to have no bearing on the haemolytic activity.

It has been said that all the 50 specimens were actively haemolytic if there was no substance present which retarded the reaction - there was one exception, however, which requires special mention. This was the urine of a case of severe anaemia associated apparently with pregnancy. The symptoms dated from the puerperium of patient's last childbirth two years previously. Several remissions had occurred, and when the patient's urine was examined for its haemolytic power the blood count was:-

R.B.C.	1,345,000
W.B.C.	3,750
Hb.	30%
C.I.	1.3

an anaemia of the pernicious type, therefore.

The urine was examined daily over a period of two weeks, -(it would have been examined further but the patient died)- and although during this time there were apparently no abnormal constituents present which would retard the haemolytic power, no degree of haemolysis was ever observed during the fortnight. The explanation may be, however, that a substance was present which was not detected, but which prevented the occurrence of haemolysis, or it may be that the haemolytic substance was absent, or present in insufficient amount to effect haemolysis, as other actively/

actively haemolytic urines are found on certain days to be non-haemolytic. This urine then, if it had been examined for a longer time, might have at some time therefore caused the reaction to take place. It is certainly exceptional, however, to find a urine without abnormal constituents to be absolutely non-haemolytic over a period such as this.

This observation is curiously just the reverse of that by McKee - who states that the more severe the degree of anaemia the more haemolytic the urine.

As a large number of urines from cases of pernicious anaemia were not available, it was impossible to arrive at any conclusion with regard to the urine of such cases, but of the urine of six cases diagnosed clinically as pernicious anaemia (one was found post-mortem not to be pernicious anaemia) two appeared to be less haemolytic than the urines from other pathological conditions tested, - no haemolysis occurring in these for a few days at a time. In one of these, which was examined when the surface tensions of a series of urines were being estimated, the surface tension reading was found to be high. As stated, however, a large number of cases would require to be available for any definite facts to be ascertained.

Considering for a moment some further clinical conditions which might be thought to have a bearing on the problem, it would have been interesting to obtain/

obtain some urines from cases of paroxysmal haemoglobinuria to test their haemolytic power. It was, however, found impossible to obtain a case of this disease. It may be mentioned that in testing such urines many difficulties will arise, for these urines are highly albuminous, which will affect the haemolysis to a marked extent, - according, as will be shown in the section of Percentage Haemolysis, - to how much haemoglobin is present and to how soon the haemolytic substance gets in contact with the cells of the blood suspension. Very great care will require, therefore to be exerted in forming any conclusion from readings obtained from urines of a disease such as this.

Ponder carried out certain experiments with a view to obtaining some indication as to the nature of this haemolytic substance in urine. As he pointed out, urines raised to a temperature of 100°C. still retain their haemolytic power. It may be noted here, however, that when non-haemolytic urines containing albumen (which prevented the reaction taking place) are boiled and the precipitate entirely filtered off, and the filtrate tested for its haemolytic power, that still no haemolysis takes place in the suspension- the haemolysin still evidently remaining in contact with the albumen. It may also be noted that although urines are filtered until clear, there is still a

positive/

positive reaction with Oliver's test, which would, if Oliver's test is reliable indicate that a certain amount of bile acids were present in the filtrate.

Ponder found that urine could be evaporated over a water-bath to dryness and the residue treated with alcohol, and if the residue was taken up by saline a haemolytic fluid was obtained.

He similarly showed that the substance is not soluble in ether, benzene or chloroform and that it is therefore fairly stable.

In 30 urines he estimated quantitatively chlorides, phosphates, oxalates, sulphur (organic, inorganic and neutral) urea, creatin, creatinin, uric acid, purin bases, hipuric acid, ammonia and total nitrogen and by less exact methods phenol, cresol, indol, indoxyl, skatol, aromatic oxyacids, leucin, cystin, tyrosin and nucleo-albumens and the investigation of all these gave no indication as to the nature of the haemolytic substance, as the excretion of none of them showed any relationship to the haemolytic activity of the urines examined.

He did find, however, a relationship between the bile acid content of urine and its haemolytic power. The test he used for the detection of bile acids was that of Oliver. Since his conclusion was that he found sufficient evidence to justify the suggestion that the haemolytic activity of urine depends on its containing minute traces of bile acids and their salts, the/

the bile acid content of a considerable number of the urines examined here were tested by Oliver's test and the results have been noted in the graphs. The only difference from Ponder's technique was that the urines examined here were not diluted 1 in 3 before testing, the method of carrying out the test, however, has been given under "Technique" in this section.

It may be added that the same peptone in the testing solution was used (Witte's) as in Ponder's experiments, although no doubt variations in the same make of peptone may be taken to occur.

In the experiments carried out, as is seen from the graphs, the bile acid content of the urine was not found to run in any degree parallel to the haemolytic activity, except in two or three cases, although the greatest accuracy was observed in taking the readings.

It must be remembered, however, that Oliver's test depends upon the precipitation of peptone in acid solution, and although no substance has yet been found to cause the test to be positive, it is possible that this precipitation may be effected by another substance which is present in urine.

Indeed, as will be shown later with sodium taurocholate in various dilutions added to a synthetic urine which was prepared, Oliver's test was negative in a dilution of 1 in 1000 sodium taurocholate, although the test was very definitely positive at a dilution of 1 in 500.

This/

This very important observation would indicate, since practically all urines gave to some extent a positive reaction with Oliver's test (a fact which has been stated by several observers) - that urine had a bile acid content, (from comparison with the average precipitate obtained with the Standard tubes, and since the precipitate is supposed to bear a definite relationship to the amount of bile acid present) of somewhere between 1 in 750 and 1 in 1000.

Dragendorf has, however, by boiling down a very large quantity of urine shown the bile acid content of urine to be nearer 1 in 100,000.

This would seem to indicate, therefore, that the precipitate obtained by Oliver's test is not due to bile acids unless these are present to a greater extent than 1 in 1000, which is not the case, and would point to the fact that Oliver's test, although a test for bile acids present to the extent of 1 in 750, is not a reliable test for bile acids present in smaller concentrations than 1 in 1000.

Moreover, as will be shown when the surface tension of this synthetic urine before and after the addition of sodium taurocholate in various dilutions was estimated, and its haemolytic activity investigated, that 0.5 cc. of the synthetic urine with 1 in 10,000 sodium taurocholate, and 0.5 cc., 0.85% NaCl only produced complete haemolysis of 1 cc. of 0.5%/

0.5% human blood suspension after 6 hours. Ponder noted this fact after 4 hours, but an important fact, as is shown later, is that little or no further haemolysis took place between 4 and 6 hours.

As will also be shown, many urines produce complete haemolysis of the suspension with only 0.1 cc. of urine taken in a shorten time even than 4 hours; while with this synthetic urine, with additions of sodium taurocholate to the amount that occurs in urine, no degree of haemolysis took place. This would support the fact that the bile acid content of the urine is not responsible for the reaction, but a fuller discussion is given in the last section.

The specific gravity has not been found to bear any relation to the haemolytic activity; neither has any drug given apparently had any connection with the phenomenon, although the urines in most cases were taken at a time when the excretion of a drug might have been expected to show some influence on the reaction if such a drug did influence it - (i.e. the urines were usually taken within an hour or two of the morning dose of the drug given).

There is nothing to indicate in these cases that age has any bearing on the amount of the haemolytic substance excreted.

S E C T I O N 2

SHOWING THE RELATIONSHIP BETWEEN

the

SURFACE TENSIONS OF URINES

and

THEIR HAEMOLYTIC ACTIVITY.

.....

THE STALAGMOMETER.

CALIBRATION of INSTRUMENT.

.....

Further Bile Acid and Specific

Gravity Readings.

_____ ..... _____

THE RELATION OF THE HAEMOLYTIC POWER OF URINE TO THE SURFACE TENSION.

There are two sets of methods adopted in finding surface tensions:-

(1) Static.- The surface tension of a fluid which is in a strictly stationary state. This is best estimated by observing the height to which the fluid rises in capillary tubes.

(2) Dynamic.- The surface tension of a fluid the surface of which is in a state of continual re-formation.

For the present problem (i.e. ascertaining the surface tension of urine) the former would be the appropriate method as the action which goes on at the surface of the red cells is essentially static.

The ideal method, then, would seem to be the estimation of the capillary rise. This presents many difficulties, and is liable to error when working with a substance such as urine, principally on account of the difficulty of thoroughly cleaning the apparatus. For this reason the surface tensions were taken by the Stalagmometer. (see diagram on page 83.) With this instrument, if a drop falls slowly the surface tension obtained is static. If falling rapidly, the surface tension obtained is a combination of static and dynamic tensions. This is really an/

an advantage because it gets over the difficulties of the dynamic method, at the same time giving an indication of the dynamic surface tension: a matter of importance since surface active substances such as many lysins tend to collect in a surface film, (e.g. take the case of saponin where there is a tendency for the saponin which has been added to go into solid surface layers).

Traube's stalagmometer consists of a bulb, an upper stem in divisions and a lower stem leading from the bulb through a capillary tube on to a dropping surface of finely polished optical glass. The lower tube may be bent or straight. The instrument used here was one with the bent type of lower tube. The method of cleaning this instrument is with 40% alcoholic potash followed by distilled water. Then by passing concentrated nitric acid through it, followed again by distilled water and finally steam may be blown through. It is then rinsed with the fluid which is to have its surface tension estimated, re-filled with the fluid and the estimation commenced.

The fluid is carefully drawn up by suction to a known point in the upper tube, at which a drop should have just fallen - or alternatively the upper meniscus is adjusted at the mark in the upper tube and then the dropping surface carefully and thoroughly dried. The number of drops is then counted until the fluid enters the lower graduated tube. The number of drops is noted when the fluid has just reached the /

the mark on the lower tube and when the next drop falls the number of divisions is counted from the known mark in this lower tube to the point at which the drop has just fallen.

For example, if the 27th drop just falls off the dropping surface at the lower mark the reading is given as 27. If, however, the 26 drops have fallen when the level of the fluid is at the lower mark and the 27th drop does not fall until 5 divisions past the mark the reading is given as 26 + 5 (= 27 drops). It is seen, therefore, that a reading of

26 = 26 drops

26 + 5 = 27 drops.

26 + 20 = 27 drops.

There are however only 20 divisions past the lower mark and 26 + 20 really is very near 26 drops as it takes 20 divisions to give the next drop. The tension then is lower at 26 + 5 than 26 + 20, although higher than 26 exactly.

The instrument must be kept absolutely vertical and absolutely steady. The temperature should be kept constant throughout. A calibration of the instrument is obtained by ascertaining the number of drops of pure distilled water delivered at 15°C. This was found to be 21 drops.- Then the surface tension in dynes/cm

$$= \frac{mg}{3.8\ R}$$

where/

where m = No. of drops.

and g = gravitation constant 981 dynes.

and R = Radius of plane of surface from which the drop falls.

Since the same instrument is used throughout S (surface tension) = K M . Where K is some constant to be determined. All that is necessary is to find the value of K and when this is known the surface tension can easily be found from the mass of a drop.

The volume of the fluid between the marks is always constant so we can put

$$S = \frac{K\,d}{n}$$ where

n = the number of drops which fall

and d = the density of the fluid.

In the case of water, as stated, 21 drops were delivered between the marks, and as the surface tension of distilled water at 15°C is known to be 72 dynes/cm.

$$72 = \frac{K}{21}, \quad \therefore \quad K = 72 \times 21 = 1512.$$

Taking Aniline as a check the surface tension of aniline at 75°C is known as

43 dynes/cm.

and/

and the density of aniline is 1.02

$$\therefore \quad 43 = \frac{K \times 1602}{36 + 15}$$

(36 + 15 = stalagmometer reading in the case of aniline.)

In order to dispose of the + 15, this is converted into fraction of a drop by finding how many divisions = 1 drop of aniline. This was found to be 13.

Then $+ 15 = 1\frac{2}{13}$ drop.

$\therefore \quad 36 + 15 = 37.15$ drops

So $43 = \frac{K \quad 1.02}{37.15} = 1597$

Considering that the rate of dropping is not the same by any means, the agreement of K is sufficiently accurate . The figure 1512 must be divided by the number of drops to obtain the surface tension is dynes/cm., e.g. if a specimen of urine gives 25 drops we find the surface tension to be 60.5 dynes/cm. An increase of 4 drops thus gives a fall of about 12 dynes/cm or 3 dynes per drop difference.

For convenience the following tables may be used, showing the relationship between the number of drops recorded and dynes/cm.

The specific gravity is greater a little than water, but this fact can be ignored owing to the fact that a figure nearer than 1 dyne/cm is not required.

The/

The Readings + may be converted into fractions of a drop by the method shown.

The first table gives the surface tensions observed in the urine by the stalagmometer at 15°C converted into dynes/cm. They are not the surface tensions at the temperature at which the urine is acting on the cells (i.e. the temperature of the incubator) and for this purpose Table II is given showing readings at 42°C. and obtained by multiplying the first figures by

$$\frac{68}{72} = 0.95$$

68 = dynes/cm. (distilled water at 42°C. known)

TABLE I.

drops	20	=	75.6 dynes/cm.
	21	=	72.0 "
	22	=	68.7 "
	23	=	65.7 "
	24	=	63.0 "
	25	=	60.4 "
	26	=	58.1 "
	27	=	56.0 "
	28	=	54.0 "
	29	=	52.1 "
	30	=	50.4 "

TABLE II.

drops	20	=	71.8 dynes/cm.
	21	=	68.4 "
	22	=	65.3 "
	23	=	63.4 "
	24	=	59.8 "
	25	=	57.4 "
	26	=	55.2 "
	27	=	53.2 "
	28	=	57.3 "
	29	=	49.5 "
	30	=	47.9 "

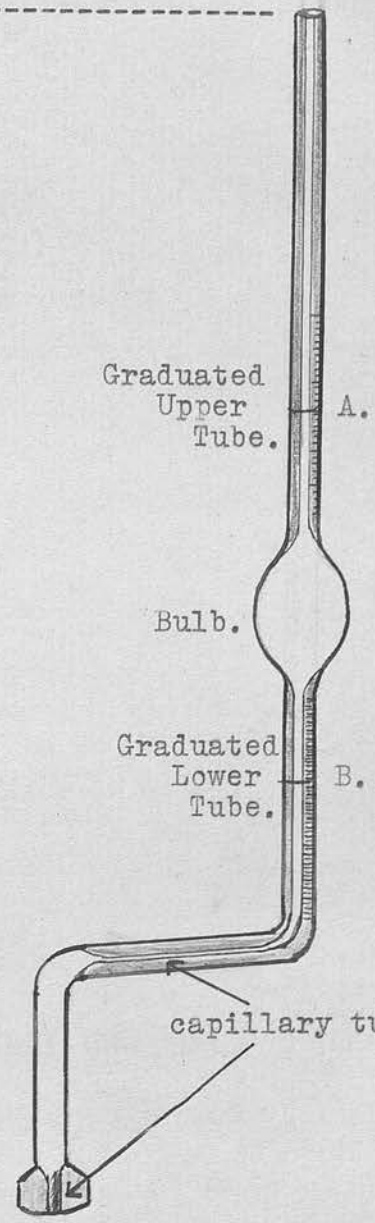
The Stalagmometer.

Method by which the surface tensions of the urines were estimated.

Temperature must be kept constant.

Instrument must be kept absolutely vertical.

Bent lower tube type of instrument.



A= Known level on upper graduated tube.
B= Mark on lower tube at which last drop is read.

It was considered necessary to estimate the surface tensions of specimens of urine which were to have their haemolytic power investigated The surface tensions were taken with the Stalagmometer and the readings of both tabulated.

A summary has been omitted with this section -the relationship between the surface activity of the urines being sufficiently shown in the tables with each of which a note is given.

The majority of these urines were collected from pathological conditions but as has been shown in the summary at the end of Section I , such urines are not more actively haemolytic than normal urine, and although a note on the diagnosis etc. was taken , this has been omitted here.

Some of the samples observed under this section are taken from apparently healthy individuals.

So far as conclusions are concerned, all that requires to be said is, that a high degree of correlation has been obtained between the surface tension readings and the degree of haemolytic power of the urine when the latter was observed for a period of about four hours, the readings being taken at intervals as stated.

It is well known, of course that the bile salts are surface active and posess haemolytic properties but so also do other haemolysins which might be reasonably expected to be found in urine. This is fully discussed on page 140.

TABLE No. 1

Amount of urine in c.c. giving complete haemolysis.
Amount of blood suspension used = 1.0 c.c..

Case No.	½ Hr.	1 Hr.	2 Hrs.	2½ Hrs.	3 Hrs.	4 Hrs.	4½ Hrs.	5 Hrs.	Stalagmometer Reading.
(1)	0	0	1.0	1.0	1.0	0.5	0.25	.25	———
(2)	0	0	0	0	0	0	0	1.0	23 + 11
(3)	0	0	0	0	0	0	0	0	25 + 19
(4)	0	0	0	0	0	0	0	0	24 + 9
(5)	0	0	0	0	0	0	1.0	0.75	24 + 16
(6)	0	0	0	0.75	0.75	.5	0.5	0.25	25 + 3
(7)	0	0	0	0	0	0	0	0	22 + 5
(8)	0	0	0.75	0.75	0.75	0.5	.25	0.25	27 + 0

From the above table it will be observed that the haemolytic activity runs absolutely parallel with the surface tension readings; the most actively haemolytic urines having the lowest surface tensions, except in the case of (4) which contains albumen and will not cause haemolysis, and (3) regarding which a note is given in the discussion.
N.B. A drop of Serum was added at 3 hrs. to the 0.25 dilution of (1) & (6) This appeared, if anything, to accelerate haemolysis already present, but on incubating overnight the 0.1 dilution of urine had haemolysed more completely than the 0.25 dilution in both cases.

Amount of urine in c.c. giving complete haemolysis at given times. 1.0 c.c. Blood suspension used.

TABLE NO 2.

Case No.	1 Hr.	2 Hrs.	3 Hrs.	4 Hrs.	5 Hrs.	Stalagmometer Reading.
(1)	0	0.5	0.5	0.25	0.25	27 + 9
(2)	0	0	0	0	0.5	25 + 20
(3)	0	0	0	0.75	0.75	26 + 17
(4)	0	0	0	0	0	27 + 20
(5)	0	0	1.0	0.75	0.5	25 + 10
(6)	0	0	1.0	0.75	0.75	26 + 7
(7)	0	0	0	0	0	24 + 18
(8)	0	1.0	1.0	1.0	0.5	26 + 12

Omit No.4 which contains albumen. No.1 is the most active haemolytically, and has the lowest surface tension. No.7 has the highest surface tension and produces no haemolysis. It is difficult to say anything definite regarding the others in the above time, but readings had to be discontinued as they cannot be taken in artificial light. No.5 appears to be active and yet have a relatively high surface tension. Similarly No.2. But these might not haemolyse further at lower dilutions than 0.5 c.c. even after several hours of further incubation. Urines such as this are shown in other tables.

Amount of urine in c.c. giving complete haemolysis.

Amount of blood suspension used = 0.5 c.c.

TABLE NO 3

Case No.	½ Hr.	1 Hr.	1½ Hrs.	2 Hrs.	2½ Hrs.	3½ Hrs.	4 Hrs.	Stalagmometer Reading.
(1)	1.0	0.75	0.75	0.5	0.5	0.25	0.25	29+3
(2)	0	0	0	0	0	0	0	23+20
(3)	0	0	0.5	0.25	0.25	0.1	0.1	26+6
(4)	0	0	0	1.0	0.75	0.75	0.5	25+17
(5)	0	0	0	0	0	0.75	0.75	22 +16

From this table again one notices the parallelism between the surface tension readings and the act - ivity of the urines (No. 2contains albumen) ex - cept in the case of No. 1. Now this urine contains bile as shown by the Nitric acid Test and Hay's Test. One would expect the bile acid content therefore to be high. From these facts then, one appreciates the very low surface tension reading, and if the bile salts were the factor in determining the haemolytic activity,this specimen should be by far the most active urine, which is not the case, although haem- olysis certainly commences first.

Dilution of urine giving complete haemolysis.

Amount of Blood Suspension used = 0.5 c.c..

TABLE NO 4

Case. No.	½ Hr.	1½ Hrs.	2 Hrs.	2½ Hrs.	3 Hrs.	3½ Hrs.	Stalagmometer Reading.
(1)	0	0.1	0.1	0.1	0.1	0.1	28 + 20
(2)	0	0.5	0.5	0.5	0.5	0.25	25 + 16
(3)	0	0	0	0	0	0	23 + 9
(4)	0	0	0	0	0	0	26 + 0
(5)	0	0.75	0.75	0.75	0.75	0.75	24 + 11
(6)	1.0	1.0	0.75	0.5	0.5	0.25	25 + 10
(7)	0	0	0	0	1.0	0.75	24 + 16
(8)	0	0.25	0.25	0.25	0.1	0.1	25 + 8

It will be seen from the above table that there is an absolute relationship between the haemolytic activity of the urines and their surface tensions, if No.4 be omitted - this specimen containing Albumen. The order from most active to least active is ;- No.1, No.8, No.6, No.2, No.5, No.7, No.3 and this is the exact order of surface tension , from lowest to highest.

The following three tables show the results obtained with urine taken from the same patients for three consecutive days - these being incub - ated with 1.0 cc. blood suspension for several hours at 42C - readings being taken hourly. In addition, the Specific Gravity of each specimen was noted, while the amount of Bile Acids pres - ent as shown by Oliver's Test is represented by by figures obtained from the standard tubes as in Section 1. The Stalagmometer Readings are also given in order to ascertain if there is any relationship between the surface tension of the urines, the amount of Bile acids present and the Specific Gravity(taken either singly or read in conjunction with each other) on the one hand,and the activity of the haemolytic substance on the other. No. 1 contains albumen however and does not produce any haemolysis and must therefore be omitted when considering the readings. No. 6, contains sugar on the first two days, and it has

been observed that this would seem to retard the haemolytic action of urine, from results noted in the case of other specimens. This will also be taken into account when interpreting the readings. TABLES 5, 6, & 7.

	1	2	3	4	5	B.A.	S.G.	Stalagmometer.
(1)	0	0	0	0	0	3	1012	24 + 15
(2)	0	0	0	1.0	0.5	4	1020	24 + 2
(3)	0	0	0	0	0	2	1012	22 + 2
(4)	0	0	0	0	0.5	3	1014	26 + 18
(5)	0	0	0	1.0	0.5	2	1012	23 + 5
(6)	0	0	0	0	0	2	1014	24 + 1
(1)	0	0	0	0	0	2	1010	23 + 15
(2)	0	0	.75	.25	0.1	3	1022	26 + 20
(3)	0	0	1.0	0.5	0.1	2	1020	26 + 16
(4)	0	0	0	0.5	0.1	2	1010	24 + 8
(5)	0	0	0	.75	0.1	3	1018	25 + 11
(6)	0	0	0	1.0	0.1	3	1026	26 + 20
(1)	0	0	0	0		2		23 + 17
(2)	0	0.5	.25	0.1		3	1020	25 + 10
(3)	0	.25	.25	0.1		3		25 + 12
(4)	0	0.5	.25	0.1		2	1016	27 + 20
(5)	0	.25	0.1	0.1		3		28 + 17
(6)	0	0	0	0.5		2	1012	23 + 20

B.A. = Amount of Bile Acids represented as described previously.

S.G. = Specific Gravity of Urine.

In these tables readings have been taken hourly. From the first table one observes ,(when Nos.(1) and (6) are omitted for reasons given) that the specimens with the lowest surface tensions are the most actively haemolytic, No(4) having the lowest surface tension. Although this urine does not commence to produce haemolysis just as quickly as Nos.(2) and (5), complete haemolysis occurs at a dilution of 0.5 c.c. in 5 hours,(i.e., just as soon as the others which commenced to produce haemolysis at lower dilutions in a shorter time.) Nos. (2) and (5) are the urines with the next lowest surface tensions and are the next most active in producing complete haemolysis of the blood suspension. No.(3) has the highest surface tension and does not cause haemolysis to take place at any dilution in 5 hours, although it contains no abnormal constituents which retard or prevent the occurrence of haemolysis.

Again in Table (2) , Specimens (2) and (3) are

most active,- indeed almost equally so - and one finds that the stalagmometer readings indicating their surface tensions, are practically the same. No.(6) has as low a surface tension as these, and is seen to be very active, although the commencement of haemolysis would appear to be retarded. This specimen contains sugar. Nos. (5) and(4) are in order less active, and also in order correspondingly higher in surface tension.

A similar definite relationship between surface tension and haemolytic activity is noted in Table (3).

With regard to the bile acids, one observes in the first place that there is very little difference in the majority of cases between the amount in these specimens, although their haemolytic activity varies to a much greater extent. From these figures one cannot state that there is any agreement with the amount of bile acids present and the haemolytic activity of the urine. More -

-over one cannot say that there is a relationship between the bile acid readings and the surface tension readings, which one would have expected when it was found that the surface tension readings corresponded with the haemolytic activity, if one ascribed that activity to the presence of bile salts in the urine.

With regard to specific gravities it is suggested from the tables that there is a tendency for the most active urines to have rather lower specific gravities, but results obtained in the case of other specimens are noted in Section 1.

TABLE No. 8

Amount of urine in c.c.,giving complete haemolysis.

Amount of blood suspension used = 0.5 c.c..

Case No.	¼ Hr.	1 Hr.	1½ Hrs.	2 Hrs.	2½ Hrs.	4 Hrs.	Stalagmometer Reading
(1)	0	0	0.75	0.5	0.5	0.1	26 + 15
(2)	0	0	0	0.25	0.25	0.25	25 + 20
(3)	0	0.5	0.25	0.1	0.1	0.1	26 + 15
(4)	0	0	0	0	0	0	25 + 0
(5)	0	0	0	0	0	0	27 + 5
(6)	0	0	0.75	0.75	0.5	0.25	25 + 20

No.(5) Contains Bile by Nitric Acid Test and has a low surface tension, but it also contains blood, the serum of which prevents haemolysis taking place. If this be omitted the urines with the lowest surface tensions are the most active. Nos. (1) and (3) have the same surface tension and are the most active, while nos. (2) and (6) have also the same surface tensions, although lower than (1) and (3), and are it would seem almost equally haemolytically active, and to a less extent than (1) and (3).

TABLE No. 9

Amount of urine in c.c. giving complete haemolysis.

Amount of blood suspension used = 0.5 c.c.

Case No.	½ Hr.	1 Hr.	1½ Hrs.	2 Hrs.	3 Hrs.	3½ Hrs.	Stalagmometer Reading.
(1)	0	0	0	0	0	0	22 + 3
(2)	0	0	0	0	0	0	23 + 11
(3)	0	0	0	0	0	0	28 + 13
(4)	0	0	0	0	0	0	22 + 20
(5)	0	0	0	0	0	0	23 + 0
(6)	0	0	0	0	0	0	23 + 11
(7)	0	0	0	0	0.75	0.75	25 + 20

A series of specimens with comparatively high surface tensions and correspondingly inactive in the production of haemolysis. No. 3 is a urine from a case of obstructive jaundice and contains bile. It has the lowest surface tension by far, but it does not produce any haemolysis. This agrees with the previous tables, and with the results obtained in Section 1. No 7 is the urine of lowest surface tension containing no abnormal substance and produces haemolysis first.

TABLE No. 10

Amount of urine in c.c. giving complete haemolysis.

Amount of blood suspension used = 0.5 c.c..

Case No.	1 Hr.	2 Hrs.	3 Hrs.	4 Hrs.	Stalagmometer Reading.
(1)	1.0	0.75	0.75	0.5	29 + 2
(2)	0	0	0	0.75	22 + 7
(3)	0	0	0	0.	22 + 7
(4)	0	0.75	0.5	0.25	26 + 19.

This table is entirely similar to the previous. The readings agree except in the case of (1) which has bile present and naturally a very high surface tension, and yet it is not the most active. One would presume that No. (6) contains more of another haemolysin which also affects the surface tension, - the very low surface tension in No. (1) being accounted for by the bile present and perhaps to some extent in the haemolysis as it is present in an amount which would conceivably produce haemolysis with the above amount of blood suspension.

Amount of urine in c.c. giving complete haemolysis

Amount of blood suspension used = 0.5 c.c..

TABLE NO 11

Case No.,	½ Hr.	1 Hr.	1½ Hrs.	2 Hrs.	2½ Hrs.	3 Hrs.	3½ Hrs.	4 Hrs.	4½ Hrs.	Stalagmometer Reading.
(1)	0	1.0	0.25	0.25	0.1	0.1	0.1	0.1	0.05	26+15
(2)	.75	.75	.75	0.5	0.5	0.5	0.5	0.5	0.5	26+5
(3)	0	0	0	0	0	0	0	0	0	24+0
(4)	Contained Albumen and was omitted.									
(5)	0	0	0	0	0	0	1.0	1.0	0.75	24+5
(6)	0	0	0	1.0	0.75	0.75	.25	.25	.25	25+11
(7)	0	0	0	0	0	0	0	0	1.0	24+0
(8)	0	0	0.75	0.5	0.5	0.25	.25	.25	.25	26+17

As it was not found practicable to take readings for e.g. ten hours owing to the difficulty of observ- the degree of haemolysis by artificial light, the above series was carried out with only half the usual amount of blood suspension. The order from lowest surface tension to highest is ;-

2 , 1 , 8 , 6 , 5 , 3 ,and 7. - while the order of most active urines to least active is ;-

1 , 2 , 8 , 6., 5 , 7 and 3. or so it would first appear, but although haemolysis occurs to a very small dilution in (1) it will be noted that haemolysis first occurs at 0.75 in (2) within ½ hour. This agrees with observations on Percentage Haemolysis,where some urines are found only to haemolyse

to e.g. 30% or 40% after many hours, although the haemolysis may commence in a short time. The haemolytic substance therefore, presumably is active but not present in sufficient amount to cause complete haemolysis of the blood suspension.

S E C T I O N 3

PERCENTAGE HAEMOLYSIS.

TECHNIQUE ADOPTED.

EXAMPLES OF PERCENTAGE HAEMOLYSIS

CURVES OBTAINED.

.....

DISCUSSION AND COMPARISON WITH PERCENTAGE HAEMOLYSIS CURVES OF OTHER KNOWN HAEMOLYSINS.

.....

PERCENTAGE HAEMOLYSIS.

On account of the difficulties which present themselves in attempting to isolate a haemolytic substance or substances from urine, - a process which introduces other factors which may in themselves produce haemolysis, or at least influence the haemolytic action, - other methods must be employed if any conclusion as to the nature of the haemolysin is to be arrived at.

It was deemed advisable, in the first place, since the presence of some haemolytic agent in urine has been demonstrated, to ascertain if this haemolysin possessed certain known characteristics peculiar to other haemolytic substances.

For this reason the Percentage Haemolysis curve of Urine was determined in order to compare it with the curves of other simple haemolysins and to find in what manner the curve for urine compared with these, and to note any differences in the curve.

The technique which was adopted is as follows:-

PERCENTAGE HAEMOLYSIS.

TECHNIQUE.

A certain definite quantity of urine was taken (the amount in each case being given on the graph). In this case, however, the urine was filtered until absolutely clear. A double strength suspension was made to allow of readings being read more accurately. For example let us say that 0.25 cc. of urine was taken. Then to twenty test-tubes were added 0.25 cc. filtered urine and 0.75 cc.- 0.85 saline and to each of these 1 cc. blood suspension (double strength, i.e. 20 cc. to 50).

The standards were made as follows for the above case (i.e. 0.25 cc. urine).

0.25 cc. urine (filtered) was added to 10 test tubes.

To the first was added 1 cc. saline.

2nd.	0.9 cc.	and	0.1 cc. Blood suspension.
	0.8 cc.		0.2 cc.
	0.7 cc.		0.3 cc.
	0.6 cc.		0.4 cc.
	0.5 cc.		0.5 cc.
	0.4 cc.		0.6 cc.
	0.3 cc.		0.7 cc.
	0.2 cc.		0.8 cc.
	0.1 cc.		0.9 cc.

0.25 cc. urine was added to each tube.

A minute amount of Saponin was added to each of these ten tubes and haemolysis was effected in a very short time.

It will thus be seen that these 10 tubes represented the degree of haemolysis of the blood suspension, - the first = no haemolysis and the tenth = 90% haemolysis and so on. These were placed in a rack.

It was found that the colors could not be accurately compared, the reason being that the standard tubes were not incubated as well - when this was done the readings were found to be facilitated.

At definite times, e.g. 15 minutes, 30 minutes, etc., a tube was taken from the incubator containing 0.25 cc. urine and 1 cc. of the suspension (i.e. one of the 20 tubes) - placed in cold water to stop or retard haemolysis, and then centrifuged for five minutes. The amount of haemoglobin which had been liberated was then compared with that of the standard tubes.

It may be said that this could have been effected more accurately with the colorimeter. This however was tried on several occasions and although theoretically it would seem an ideal method for comparison, in the case of urines, it was not found practicable. It necessitated a very large number of standard tubes being prepared as each standard required to be in the incubator the same length of time as the urine tubes which were to be tested. Even on this method being tried the colours were found not to match very well owing, presumably, to the formation of haematins from the haemoglobin by the substances in the urine. This adds yellow to the red and the colorimeter method/

method had to be abandoned. As stated, however, the alternative method which was devised was found quite accurate.

A few words of explanation are given with each graph.

.....

By this method, then, the reaction is stopped after it has proceeded a certain length, the stopping being obtained by cooling, which markedly reduces the rapidity of haemolytic reactions; the intact cells are centrifuged off and the amount of haemoglobin in the supernatent fluid estimated by comparison against standard tubes.

Cooling however, does not stop the reaction - it only retards it. This would be a disadvantage where extremely active haemolysins are used, or haemolytic agents in high concentrations. Also the centrifuging will cause those cells which have been weakened by the haemolytic agent to haemolyse.

With a substance such as urine these are by no means serious disadvantages although they must be taken into account. The comparative inactivity due no doubt to the low concentration in the amounts used in the experiments, makes this method more applicable than might be at first supposed.

Brooks in his paper on the Theory of the mechanism of Haemolysis and Similar Processes, has stated that the apparent course of such processes as haemolysis is determined by the rate of change in the number of living cells which have undergone some definite alteration such as laking and loss of viability.

His paper deals with the physico-chemical process or groups of processes leading to death, laking and similar effects in determining progressive changes in the number of individual cells succumbing in successive units of time to the deleterious agent. He terms the physico-chemical process in the protoplasm the "fundamental process", and the time curve of any process like haemolysis, the "course of the process".

He points out that if erythrocytes are suspended in an indifferent medium and are subjected to a brief radiation from a mercury vapour arc in quartz, or if they are suspended in an appropriate dilution of serum acting in conjunction with a specific antibody, there ensues a gradual liberation of haemoglobin.

In both cases the process begins at a rather slow rate which gradually increases, passes through a maximum and then gradually decreases until it becomes comparable with the rate of spontaneous laking. This phenomenon will be shown to take place when urine is/

is taken as the substance in which the haemolytic agent is contained, and to the action of which the erythrocytes are subjected, suspended in 0.85% NaCl, - an indifferent medium, as shown by the controls.

Plotting as ordinates the percentage of haemoglobin liberated and as abscissae the time at which the readings were taken, asymmetrical sigmoid curves were obtained, similar to those which Brooks describes.

He explains the gradual retardation and final apparent equilibrium as either due to exhaustion or inactivation of the lytic agent, or if the concentration of the lytic agent is increased above that which is essential for complete haemolysis in a few hours, the process ceases because of the exhaustion of the supply of cells.

Brooks has shown that after enumeration of the red blood corpuscles visible in a given volume of suspension at various stages of haemolysis, the amount of haemoglobin which is liberated is almost proportional to the number of cells which have lost their pigment.

The technique adopted in endeavouring to obtain a percentage haemolysis curve for urine is based on the above observation, and on the fact, as Brooks showed, that the course of haemolysis depends on the relative number of red blood corpuscles having in different degrees the power to withstand the action of/

of the haemolysin - for since the amount of haemoglobin liberated is proportional to the number of erythrocytes which have disappeared, the observed course of haemolysis must be regarded as the summation of the laking of individual cells at varying times after they are subjected to the condition leading to haemolysis.

Again Brooks points out that a few relatively fragile cells are laked almost immediately, the resistant ones surviving for a very much longer period - but most of the cells succumb during an intermediate period when the observed rate of haemolysis is at a maximum.

In the second group of curves obtained with urine as the lytic agent, it will be noticed that haemolysis is not complete. This is due to a deficiency in the amount of haemolytic substance no doubt, but these curves show that haemolysis in each case has reached this maximum rate.

When haemoglobin begins to diffuse from a given erythrocyte the process, according to Brooks, is quickly completed, i.e. it may be ordinarily regarded as instantaneous.- Any increase in the relative number of the more fragile cells accelerating the reaction, any decrease giving a corresponding retardation.

The method adopted in the case of urine in expressing graphically the progress of the haemolysis was/

was by the time curve where the ordinates are proportional to the total number of cells laked, and the rate of haemolysis is therefore represented by the slope of the curve - the steeper the curve the more cells succumbing per unit of time to the haemolytic substance contained in the urine. The first two curves of complete haemolysis given are typical examples of the complete percentage haemolysis curve of urine, the partial haemolysis curves are typical examples obtained where the haemolytic agent was present in insufficient amount. It must be pointed out that curves were not found to differ in type with the urine from several different pathological conditions and these did not vary from the curve obtained when normal urine was used. The amounts of urine required to effect the haemolysis are given with the curves, similarly the amount and strength of the suspension.

Examples of the complete curve are given where the haemolysis did not take a longer time than a few hours, as after that time readings were more difficult to interpret in some cases; but apart from this it must be remembered that other factors may come into play - indeed in the case of urines which only haemolysed the same amount of suspension after e.g. 20 hours. - it is by no means certain that the same haemolytic agent is responsible as in these urines which/

which cause haemolysis in an hour or two. As the Standard tubes had to be placed in the Incubator as well, it will be readily understood that a long incubation period diminishes greatly the differences in colour between these, and introduces a source of error.

When working with a substance such as urine, it is difficult to obtain a frequency curve as it has been shown from results obtained under "Technique" in Section I - and confirmed by observations by McKee, that the same amount of the same urine may in a given time of incubation at the same temperature cause haemolysis to a slightly different degree in the two cases, although the amount of blood suspension in each case was equal in amount and of the same strength - i.e. that the same quantities of the same urine do not evidently necessarily contain exactly the same amount of the haemolytic substance.

Although readings were taken for a frequency curve the method was open to too many fallacies and thus frequency curves have been omitted.

On the other hand Brooks concluded that haemolysis is largely dependent upon variations in resistance among the different individuals, and it may be, therefore, that this difference is shown in two equal amounts of blood suspension which would also account for the variations just described and also for

variations/

variations in haemolytic activity in the same amounts of the same urine described in other Sections.

Ponder has described important and typical percentage haemolyses curves obtained with various haemolytic substances under various conditions. He has shown in full the relation between percentage haemolysis and time in the case of three haemolytic substances

Sodium hydroxide

Saponin

and Sodium taurocholate.

The sodium hydroxide curve he obtained exhibits a distinct latent period when no haemolysis occurs, followed by the beginning of the reaction resulting in haemolysis which is described by a sigmoid curve which is almost symmetrical - the deviation from symmetry being so small as to fall within the range of experimental error. The median of the sodium hydrate curve nearly coincides with the ordinate erected at a point half way between the commencement of haemolysis and its completion.

Now the curve obtained with urine is not a symmetrical curve like the above, and Ponder has pointed out that the main difficulty in attempting to ascertain the nature of the haemolytic reaction lies in the fact, that, in the case of haemolytic agents usually employed for such experiments the curve/

curve relating to percentage haemolysis is asymmetrical. The percentage curve of urine then, possesses the characteristics of the curves of most of the commoner haemolytic substances, but even with these asymmetrical curves certain variations occur as Ponder has observed, and the difficulty in likening the asymmetrical percentage curve of one haemolytic substance to the asymmetrical percentage haemolysis curve of another cannot be underrated.

It is therefore with caution that the curves obtained from urine are interpreted as not bearing the same characteristics of the sodium taurocholate curve obtained by Ponder, although this is certainly suggested.

In these asymmetrical curves the number of red cells destroyed in the first half of the reaction is greater than that in the second half. Ponder explains this by stating that there is direct evidence to show that the process of haemolysis results in the liberation of substances from the cell which have a retarding effect on the fundamental reaction, together with the fact that as lysis proceeds the amount of free lysin gets less and less.

He obtained with sodium taurocholate an asymmetrical curve where the latter part of the haemolytic action would appear to beslower than in the case of urine, although the first half of the two curves do not disagree; and as Ponder has proved that the action/

action of serum proteins and also haemoglobins retard sodium taurocholate haemolysis if added before the haemolytic substance is in contact with the cells, although curious enough an acceleration is produced of nearly all cases if the serum is added after partial haemolysis has been effected by the lytic agent already in contact with the cells, The result of addition of serum etc. to suspension partially haemolysed by urine was found to be a definite retardation - this is what occurs with haemolytic agents such as saponin. (Experiments, however, with urines containing blood (serum) and where therefore the haemolytic agent was in contact with the cells as soon as the serum - the retardation was always apparent.).

When serum was added to tubes containing blood suspension which had been partially haemolysed by the haemolytic substance of urine this retardation was apparent - e.g. if the serum was added to a tube containing 0.25 cc. of urine, it would be found that while in the 0.1.cc. dilution,the suspension had undergone complete haemolysis, the 0.25 cc. dilution had evidently remained partially haemolysed to the same extent as before the serum was added.- The fact to be determined was whether there was a retardation, and not to what extent the retardation occurred, so that the amounts of liberated haemoglobin before and after/

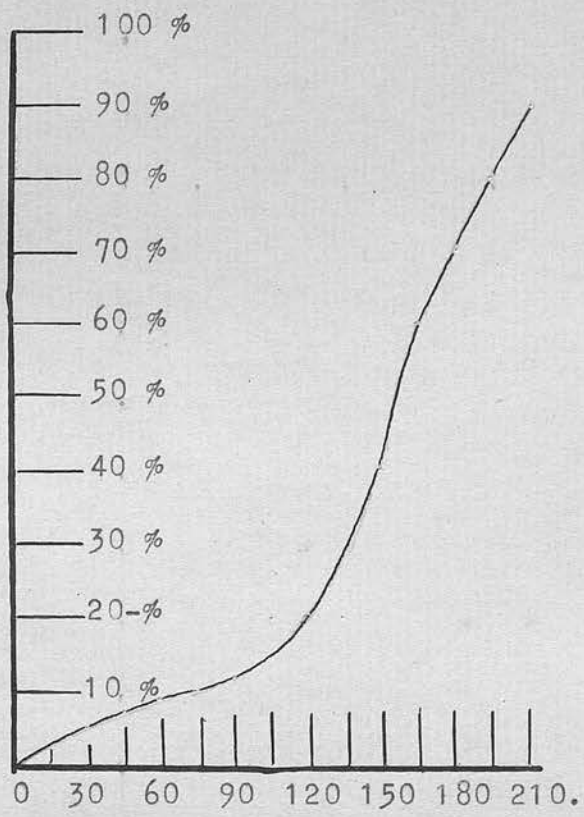
after the addition of serum were not estimated.

It might be assumed, therefore, that as the lysis with urine was not accelerated by the addition of serum, that the contained haemolytic substance with other corroborating evidence is not the sodium taurocholate content, but a haemolytic substance allied to saponin. The same might also be assumed from the different type of skewness in the curve obtained from the experiments with urine and those with sodium taurocholate. In the latter case, however, great care and experience are required in arriving at conclusions which rest on so delicate a point. One definite fact is however clear - that the percentage haemolysis curve obtained from urine possesses the characteristics and peculiarities of some of the well-known haemolytic substances, and it is suggested that the percentage haemolysis curve differs from that obtained from sodium taurocholate. Another fact is of importance, that the curve obtained with normal urine does not differ from that obtained with urine from the unselected morbid states which was tested.

CURVE of PERCENTAGE HAEMOLYSIS.

0.5 c.c. urine and 0.5 c.c. 0.85 % NaCl with the addition of 2.0 c.c. Human Blood Suspension, 0.5 %.

Controls. No haemolysis took place in 3½ hours at 42 C. with 1.0 c.c. 0.85 % NaCl. No haemolysis occurred either with 0.5 c.c. NaCl 0.85 % & 0.5c.c. water in the above time. Readings were taken at 15 minute intervals.



Ordinate = Percentage Haemolysis.

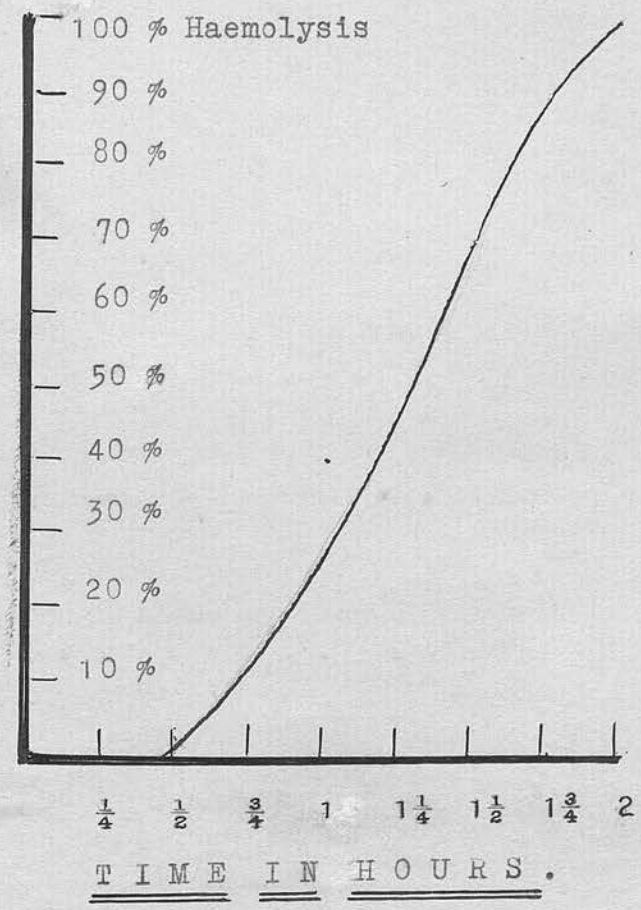
Abscissa = Time in Minutes.

Urine . Case No.

Reaction = Acid,
Specific Gravity = 1012,
No abnormal constituents.

PERCENTAGE
HAEMOLYSIS.

This urine, the percentage haemolysis curve of which is given below, is extremely active - complete haemolysis being effected at a dilution of 0.25 c.c with 0.75 c.c 0.85 NaCl, in 2 hours.

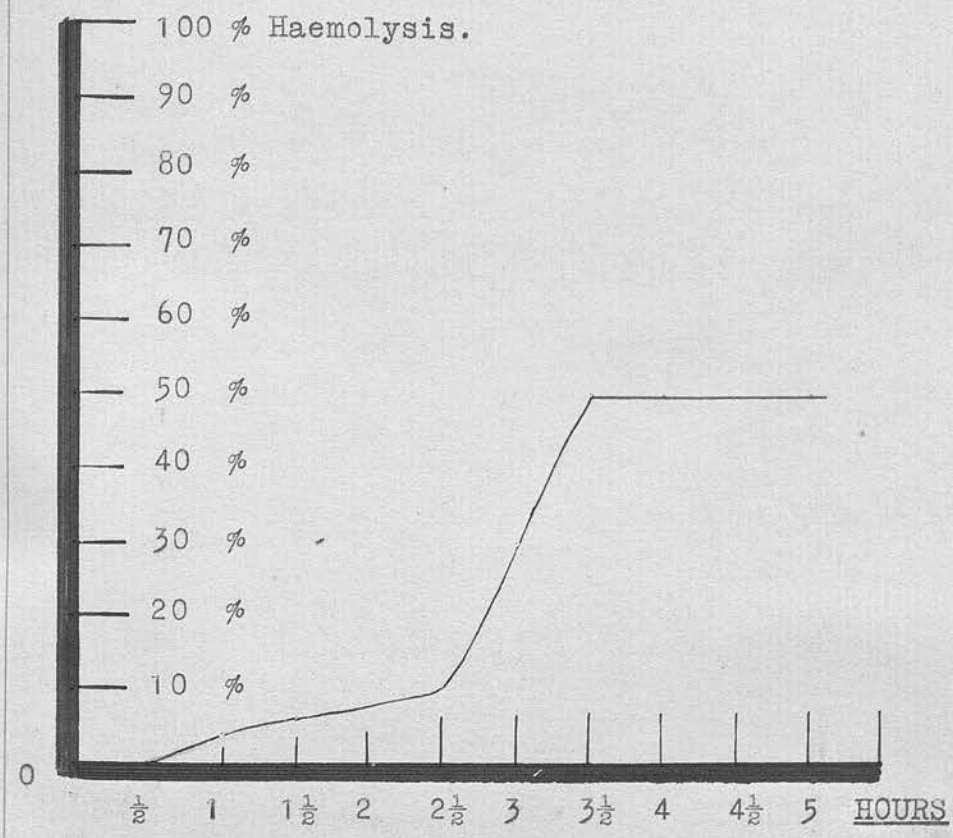


Controls - No haemolysis took place.

PERCENTAGE HAEMOLYSIS.

Quantity of urine taken in each tube = 0.5 c.c.

(Urine of Case No. Rheumatism , Acute.)



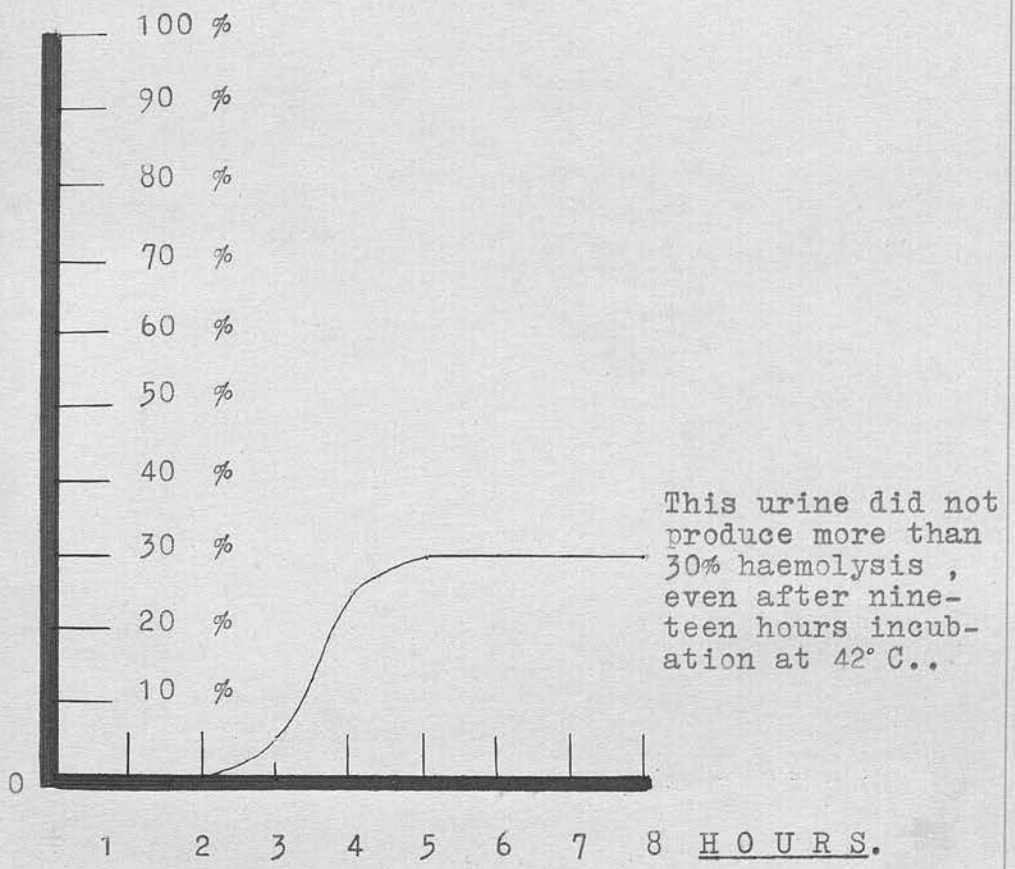
Controls;- No Haemolysis took place.

From the above curve one observes that at the commencement the type of curve is similar to those obtained where complete haemolysis was produced. Here, however, haemolysis stops abruptly at 50 % and does not proceed further even after several hours of further incubation at 42°C. There is therefore presumably only sufficient of the haemolysin contained in 0.5 c.c.urine to effect partial haemolysis.

Urine from a healthy adult. Normal specimen

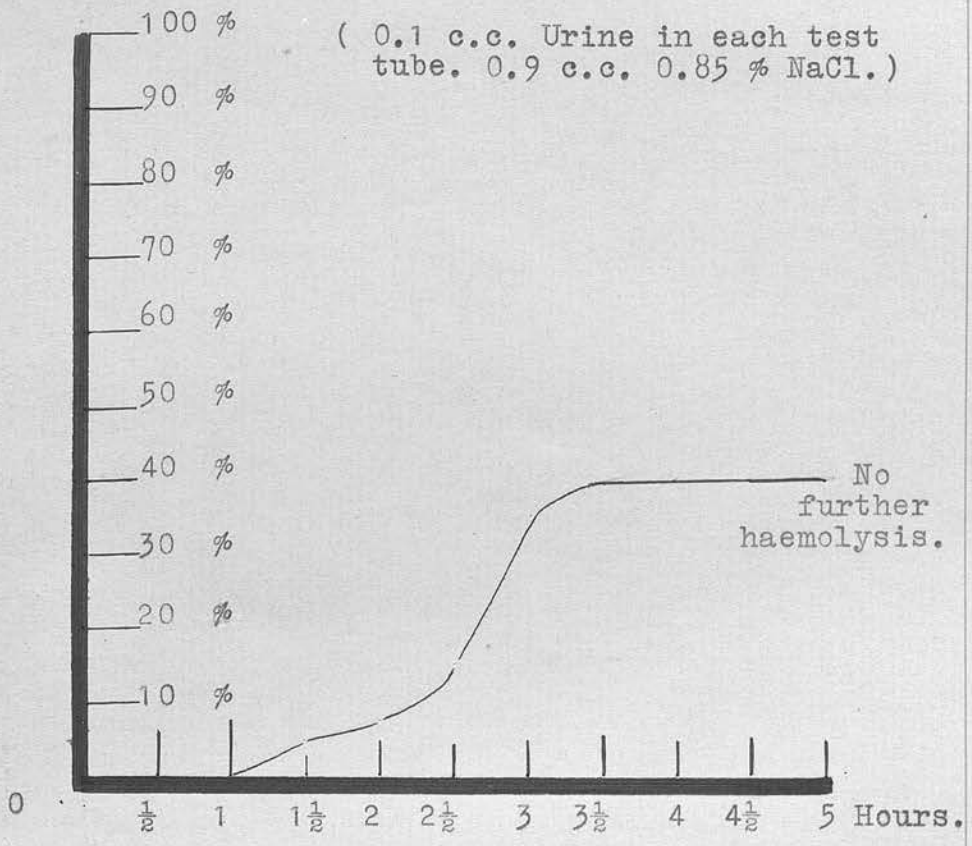
PERCENTAGE HAEMOLYSIS.

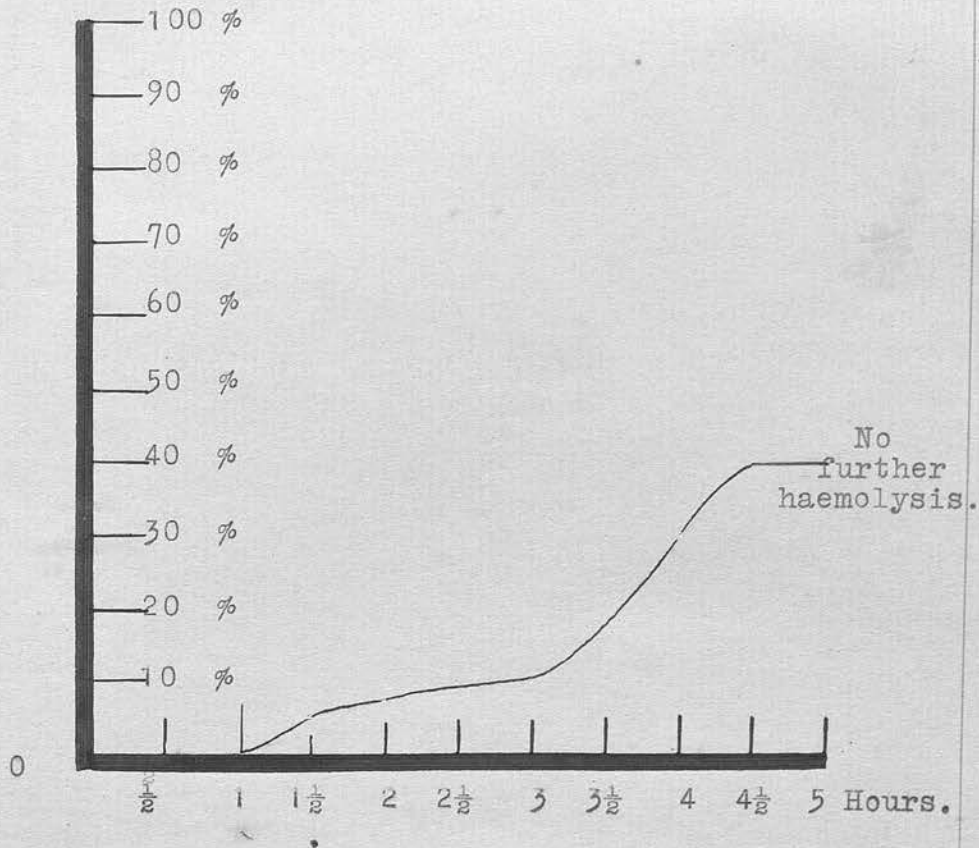
Quantity of urine taken in each tube = 1.0 c.c.
i.e., undiluted with 0.85 % NaCl.



In the above curve we notice the beginning as before; also the abrupt rise. When haemolysis reaches 25 % however, the process would appear to have almost stopped and when 30 % is reached no further action takes place.

Controls with this experiment were again negative.





Two graphs showing that only enough of the haemolytic substance was contained in 0.1 c.c. to produce 40% haemolysis.

S E C T I O N 4

RESISTANCE SERIES.

ERYTHROCYTES OF VARIOUS ANIMALS TESTED

AGAINST

THE HAEMOLYTIC ACTION OF URINE.

RESULTS OBTAINED

WITH

SHORT DISCUSSION ON

THESE.

ERYTHROCYTES OF VARIOUS ANIMALS TESTED AGAINST THE HAEMOLYTIC ACTION OF URINE.

Considering further the characteristics of the better known haemolysins, it is found that the series of Ryvosh is concerned with the order in which the erythrocytes of the mammalia may be placed with respect to the very active haemolysin saponin, and to hypotonic saline respectively.

It is interesting, therefore, and not irrelevant, to find the order in which the red blood cells of certain of the mammalia are placed when urine, (both from morbid states and from healthy individuals) with its contained haemolytic substance is used in place of saponin.

Especially is this the case when the percentage haemolysis curves of urine (normal and urine from pathological conditions) were suggestive of the percentage haemolysis curves of saponin, and as this observation is supported by the fact that serum added, when the haemolytic substance is first allowed to be in contact with the cells of the suspension, does not produce an acceleration of the reaction as is found with sodium taurocholate in nearly all cases, but a retardation which is found in the majority of cases with/

with the saponin series.

Looking at the series of Ryvosh from another point of view, it was thought advisable to carry out a few experiments such as this in order to find if the order of resistance of the erythrocytes was that which Ryvosh obtained with hypotonic saline, as hypotonicity is a factor which may theoretically be suggested as a cause - or a factor which may greatly influence - the haemolytic reaction obtained with urine. A fuller note on tonicity, however, is given elsewhere.

Using saponin as a haemolytic agent, Ryvosh obtained the following order of resistance, from the most resistant red cells to the least resistant:- sheep, goat, ox, cat, mouse, pig, grey rat, dog, white rat, rabbit, guinea pig.

The order obtained for hypotonic saline was the reverse, (i.e. guinea pig, was the most resistant) except in the case of the rabbit which was out of place in the series.

In the case of urine it was unnecessary to test the erythrocytes of all these animals as much can be learned by testing the resistance of a few. Moreover the difficulty of obtaining the blood of most of these animals on one day could not be overcome, as the same specimen of urine had to be used with the different suspensions at the same time, i.e. other substances may be kept for a period of hours and days for testing but/

but this cannot be done in working with a substance like urine, and another specimen from the same individual will not show the same haemolytic activity as has been shown.

The strength and amount of suspension used in each series was the same.

Tables showing the results obtained are given at the end of this section.

It need not be emphasized that accurary was essential.

It was learned during these experiments with the different blood suspensions, that the more dilute the blood suspensions the more accurate were the results obtained.

Ponder in modifying the method of Ryvosh pointed out that Ryvosh added defibrinated blood to his solutions of NaCl and of saponin, and the haemolytic systems, therefore, contained serum which he (Ponder) has shown to inhibit saponin haemolysis.

The method of preparing the suspensions varies; that used here was that of Ryvosh where 1 cc. contains cells from a constant volume of blood. The important fact is, however, that the more dilute the suspension the more accurate are the readings obtained.

The order of resistance of the red cells obtained with urine as the lytic agent was from most resistant to/

to least resistant:-

Sheep, Ox, Rabbit, and Man.

It is unnecessary for the present problem to go into the theories as to why this variation in resistance occurs, but it may be said that Ponder has suggested that the resistance of cells to saponin is principally determined by the protein content of the cell, exclusive of haemoglobin. He confirms, on the whole, the results of Ryvosh.

What is seen from these experiments with urine is that the results agree with results obtained from the saponin series and not for those obtained with hypotonic saline.

As was suggested previously it is uncertain whether the same haemolytic substance was responsible for the haemolytic action of urine, when haemolysis was not effected for a long period of incubation, e.g. 20 hours, and when the haemolysis was much more rapidly produced; it may be stated that no difference was found in the order of resistance with either of these types of urine. On the other hand more than one haemolysin may produce the same order of resistance. Neither was a difference in the series obtained with the urine from healthy individuals and that from certain morbid conditions.

Results obtained by Kofler and Lazar may be very briefly referred to here. They claim to show that the resistance series of saponin is different to that of digitoxin, /

digitoxin, sapotoxin, senegin and other saponins, and that these have different series one from the other.

If this were an established fact the order of resistance for the lysin contained in urine might have been fully worked out and by this means it would have been possible to eliminate certain members of the saponin group as not being responsible.

There are certain fallacies, however, which make this valueless. Kofler and Lazar used suspensions which were unequal in strength, and are too concentrated. Moreover they place the erythrocytes of one animal before that of another, if the resistance of both is the same, for no other reason than that it occurs first with the original substance tested (i.e. saponin).

Apart from saying that this unknown haemolysin contained in urine gives a resistance series corresponding with that of saponin, it cannot be identified by this method.

The tables below show the greater resistance of the erythrocytes of the Ox to the haemolytic action of urine. The blood suspension used was prepared in a similar manner to that of the human blood suspen - sion hitherto used, being 0.5 %. The two sets of urines were incubated at 42 C for 4 hours together, readings being taken at intervals as noted. 0.5c.c. Ox blood suspension was added to one set of test tubes containing the usual dilutions of urine; 0.5 c.c. human blood suspension to the other.

Table No.1 = Human Blood Suspension.

	½	1	1½	2	2½	3	4 Hours.
(1)	0	1.0	1.0	0.75	0.75	0.75	0.1
(2)	0	0	0	0	0	1.0	0.5
(3)	0	0.5	0.25	0.25	0.25	0.1	0.1
(4)	0	0.5	0.5	0.5	0.25	0.25	0.25

Table No.2 = Ox Blood Suspension.

(1)	0	0	0	0	0	0	0
(2)	0	0	0	0	0	0	0
(3)	0	0	1.0	0.75	0.5	0.25	0.25
(4)	0	0.75	0.75	0.75	0.75	0.75	0.5.

Resistance of the erythrocytes of various animals to the haemolytic activity of normal urine. The following is an example of a very inactive urine, which points out the fallacy, however, of only observing such urines for short periods.

These readings were only obtained after 20 hours incubation at 42° C.

Human Blood Suspension.	Complete Haemolysis.	at	0.25 cc.
Ox Blood Suspension.	"	"	0.5 cc.
Rabbit Blood Suspension.	"	"	0.1 cc.
Sheep Blood Suspension.	No haemolysis occurred.		

The Suspension in each case was of 0.5 % strength, and 0.5 c.c. was used for each dilution of urine.

It will be seen from the above experiments that the series obtained is, in order of most resistant red blood cells to least resistant ;-

Sheep,

Ox,

Man,

Rabbit.

.Further examples of the difference in resistance of the red cells of various animals to this haemolytic power of urine, - in both these cases nomal urine (urine from healthy individuals), was used. 1.0 c.c. undiluted with 0.85 % NaCl. with 0.5c.c. of a 0.5 blood suspension in each case. Also diluted specimens as formerly.

Human blood suspension.	Complete Haemolysis at	1.0 cc. in 3 hrs.
Ox blood suspension.	No Haemolysis	in 3 hrs.
Rabbit blood suspension.	Complete Haemolysis at	1.0 cc. in 3 hrs.
Sheep blood suspension.	No Haemolysis	in 3 hrs.

The human red cells and those of the rabbit are equally resistant while those of the ox and sheep are most resistant , not being laked in the 3 hours.

(2nd Specimen.)

Human.	Complete haemolysis at			0.5 cc.	in 3 hrs.
Ox.	"	"	"	1.0 cc.	" 3 hrs.
Rabbit.	"	"	"	0.5 cc.	" 3 hrs,
Sheep.	Haemolysis just commenced at			1.0"	3 hrs.

The order of resistance here, commencing with the most resistant, is ;-

Sheep,

Ox,

Human and Rabbit.

Further Examples.

Normal Urine. Incubation of 4 hours at 42° C.

0.5 % Suspension in each case. 0.5 c.c. used.

Human blood.	complete	haemolysis	at	0.1 c.c.
Horse blood.	"	"	"	0.5 c.c.
Rabbit blood	"	"	"	0.1 c.c.
Ox blood.	"	"	"	0.75c.c.
Sheep blood.	"	"	"	0.25c.c.

In this case the series obtained is, in order of most resistant cells to least resistant ;-

Ox,
Horse,
Sheep,
Rabbit & Man.

The suspension in the case of the horse blood above was too concentrated and has resulted in this blood being out of place in the series.

S E C T I O N 5

VARIOUS EXPERIMENTS.

HAEMOLYTIC ACTIVITY

of

URINE OBSERVED

Before and after exercise.

.....

Before and after drinking large quantities of water.

.....

Experiments with decomposing urine.

.....

Changes in cells which occur during haemolysis.

Experiments with urine of animals.

.....

Experiments with urine from normal individuals before and after drinking a large quantity of water.

The following experiments show the difference in the haemolytic power of urine before and after drinking large quantities of water. They were carried out as follows;-

Several persons whose urine contained no abnormal constituents, passed urine - a sample of which was taken. They then emptied the bladder completely. Having done this each person proceeded to drink half a litre of tepid water. After half an hour a sample of the next urine passed was collected.

The two specimens in each case were tested in the usual way , being incubated for four hours, at the usual temperature, 42 C.

The two sets of tubes were, of course, put up together at the same time in the incubator and were read at the same time.

The following results were obtained ;-

	Specimen of urine before drinking the water.	Specimen of urine after half a litre of water
	Complete Haemolysis occurred at ;-	Complete Haemolysis occurred at ;-
(1)	0.1 c.c.	0.1 c.c.
(2)	No haemolysis.	No haemolysis.
(3)	NO haemolysis.	0.1 c.c.
(4)	No haemolysis.	0.1 c.c.
(5)	No haemolysis.	0.1 c.c.
(6)	0.1 c.c.	0.1 c.c.
(7)	0.5 c.c.	0.1 c.c.
(8)	0.1 c.c.	0.25 c.c.
(9)	1.0 c.c.	0.05 c.c.
(10)	No haemolysis.	0.05 c.c.
(11)	0.5 c.c.	No haemolysis.
(12)	No haemolysis.	No haemolysis.
(13)	0.75 c.c.	0.5 c.c.

From these readings - which are some of the results obtained when the urine from normal individuals was examined before and after drinking half a litre of tepid water - we see that haemolysis in almost every case occurs much more readily; the explanation being the difference in tonicity which after all is what is to be expected. In the very few cases where the urine does not appear to be more actively haemolytic after drinking the water, account must be taken of the fact that the person may not have emptied the bladder quite completely in the first instance; also that the production of diuresis (giving the difference in tonicity to the urine) varies in time in individual cases, and in these experiments the second specimen was collected ½ hour after drinking the water.

Although McKee in his paper states that he never observed any haemolytic action with the urine of normal individuals, he goes on to say that he conducted a routine examination of the urines of all new patients entering hospital and he regards these with importance, his reason being that he found that the urine obtained on the first day following the patient's admission was often haemolytic, although never again during a stay of days or weeks in hospital.

Now it has been shown in these experiments with normal urine, before and after the patient has been given/

given half a litre of water to drink, that the urine is, in the majority of cases after drinking the water, much more actively haemolytic due more than probably to the difference in tonicity.

This is what might be expected from persons just admitted to hospital - diuresis - which in fact, very often occurs and might be suggested as an explanation of McKee's observations.

It will be asked, however, why McKee did not find this haemolytic action on the days following the patient's entry, and the answer to such a question would seem to lie in the fact that the period of incubation adopted by McKee was 2½ hours at 37°C. In the experiments given here it has been shown on many occasions that while a urine may be easily classed as non-haemolytic when only observed after 2½ hours, if that specimen be observed for a further period haemolysis may be easily found to occur. This is one important difference in the technique adopted here, and that followed by McKee. In some cases urines required to be observed for 20 hours before their haemolytic power was apparent, although in the majority of cases a much shorter period was sufficient, e.g. 4 hours. Nevertheless a marked difference in results obtained is apparent when urines are observed for 2½ hours on the one hand and 4 hours on the other. Again the necessity of observing/

observing the urines at intervals during this period has been emphasised when endeavouring to find some factor which may have a relationship with the haemolytic activity. This is brought out in the surface tension readings taken under another section.

Although it will be shown that tonicity has no bearing on the phenomenon ordinarily observed, it may be mentioned that the samples of urine obtained here were taken first thing in the morning, and taken at the same time each morning. Moreover any factor which might be expected to alter the tonicity to any extent was noted.

Lastly, it may be stated that specimens of urine were not taken for examination until patients had been in hospital for a day or two.

As so many factors have to be considered when dealing with a substance such as urine, it was thought that attentions to technique such as these would allow readings obtained to be more easily interpreted.

CHANGES IN FORM OF CELLS DURING HAEMOLYSIS.

Observations were carried out on the change of form which cells undergo during haemolysis with a view to finding how these changes correspond to those changes already described by Ponder for saponin and other haemolysins.

The cells were observed under the microscope in the usual way, the observations being carried out in an incubation room at 37°C., the temperature necessary in order that lysis may proceed.

The changes which occur take place in a definite order.

Cells in suspension in NaCl present typical Goughian form, appearing as perfect spheres whose volume is the same as that of cells in the more familiar discoid form. Close examination shows the surface of the cell to be covered with minute crenations.

On addition of the lytic urine the form of the cell changes quickly, the Goughian form being converted into the discoid form usually with coarse crenations. These may be due to the fact that the tonicity of the system is not perfectly maintained after the addition of urine. In this state the cells remain until the time of haemolysis approaches.

Just before lysis the form of the cell alters

and/

and the spherical form is again assumed and the cells appear as glistening spherical bodies on the surface of which no crenations are visible, thus distinguishing them from the Goughian form.

The haemoglobin is then liberated; the cells become pale and ultimately invisible except for the ring which appears to correspond with the now empty cell membrane. In time (hours) these "ghosts" disintegrate.

These changes are identical with those observed by Ponder in the case of haemolysis by saponin and bile salts.

These observations, therefore, throw no light on the nature of the haemolytic substance contained in urine, for they occur with most of the simple haemolysins such as saponin, sodium taurocholate, sodium glycocholate and the soaps.

Some Readings obtained from

Urines Examined Before and After Exercise

	Before. Complete Haemolysis at;-	After. Complete Haemolysis at;-
	(After 4 hours incubation at 42° C.)	
(1)	0.25 c.c.	0.5 c.c.
(2)	No Haemolysis	No Haemolysis
(3)	0.75 c.c.	1.0 c.c.
(4)	0.1 c.c.	0.1 c.c.
(5)	0.25 c.c.	0.25 c.c.
(6)	0.1 c.c.	0.1 c.c.
(7)	0.1 c.c.	0.1 c.c.
(8)	No Haemolysis	No Haemolysis.
(9)	No Haemolysis	No Haemolysis.
	Readings of (8) & (9) after 20 hours incubation =	
	0.25 c.c.	0.25 c.c.
	0.75 c.c.	No Haemolysis
(10)	0.5 c.c.	0.1 c.c.
(11)	0.25 c.c.	0.25 c.c.
(12)	No Haemolysis	No Haemolysis.

Although only the readings after 4 hours are given here, readings at varying intervals were taken but no purpose is served by tabulating these. As stated the above are some of the readings and show that in most cases the haemolytic activity is unaltered after exercise. Some specimens examined however showed first a slight increase in activity after exercise only to be later followed by a slight retardation. Any increase or decrease observed was relatively

small, the majority of urines haemolysing the blood suspension to the same extent before and after.

It may be mentioned however that in those urines which showed any slight change in haemolytic activity, the bile acid content taken as before by Oliver's Test, was not found to be altered in the two specimens.

Obviously unless the difference in activity before and after exercise were marked it could not be taken into account as such slight changes such as have been described can be accounted for by the fact that two separate samples were taken and it would be erroneous to presume that exactly the same amount of the haemolytic substance would be excreted in each specimen.

Moreover it has been pointed out in Section I that at times urines were encountered where the same amounts of the same urine incubated for an identical period did not produce exactly the same degree of haemolysis. McKee also drew attention to this fact in his paper.

The form of exercise adopted was running up and down two flights of stairs - short of fatigue.

OBSERVATIONS ON THE HAEMOLYTIC ACTIVITY OF DECOMPOSING URINES.

This question of decomposing urine seems to arise when the question of haemolytic activity is considered, and curiously enough it plays little if any part in influencing the phenomenon.

McKee states in his paper that the haemolytic action does not depend upon the acidity or alkalinity alone, although he concluded that the majority of the most powerfully haemolytic urines are alkaline in reaction. This observation was not confirmed here.

He also pointed out that acid urines tend to lose their haemolytic power on standing twenty-four to forty-eight hours; in fact he notes the fact that urines seem to be of two orders - one which is alkaline on passage or becomes so in a short time - another acid group which remains acid despite bacterial contamination and fermentation.

The urines examined here were unselected as regards their reaction, but if found to be haemolytic, these were kept at room temperature for varying periods - 24, 48 or 72 hours, after which period their haemolytic action on the same amount of blood suspension was again tested. The readings need not be given. All that requires to be said is that in some the/

the reaction was slightly retarded, and in others a slight acceleration was noted. This difference, however, was slight and as we have seen such differences may occur from two specimens of the same sample of urine incubated for an identical period, obviously no stress could be laid on an observation such as this. The difference in haemolytic activity, had it been marked, would have been worthy of note. It is presumed, therefore, that decomposition of urine has no bearing on the problem as ordinarily observed.

Urine from Animals.

It may be mentioned here that this haemolytic activity was also found in urine from the Rabbit, Goat, Frog, Cat. The activity was apparently much the same as was obtained with human urine and for this reason this investigation was not carried further.

Some Common Substances influencing the Haemolytic Reaction.

(1) Albumen.

A series of twelve urines containing varying amounts of albumen, were tested for their haemolytic power over a period of several days. In only one was any haemolytic power apparent and in general it may be said that urines containing as much albumen as 1 Gramme per litre will be found non-haemolytic. If these urines be boiled and the precipitate carefully filtered off still no haemolysis of the blood suspension occurs, the haemolytic substance evidently remaining in combination with the albumen.

(2) Blood.

Specimens containing blood according to the Guiac Test will be found to be inactive as regards haemolytic power or at least of very low haemolytic power. This observation merely confirms Ponder's work on the investigations on the inhibitory effect of serum with various haemolytic substances.

(3) Pus.

Urines containing pus have also been found to be non-haemolytic or very inactive

(4) Sugar.

Urines containing sugar would in some cases appear to be less actively haemolytic.

(5) Sodium Bicarbonate.

Ponder and McKee have already stated that the addition of this substance exerts an inhibitory influence on haemolysis produced by urine. McKee has noted that if this substance be given by the mouth to persons whose urine he found to be haemolytic, the haemolytic power is lost. It is found here that an inhibitory effect is suggested if sodium bicarbonate be given by the mouth.

TONICITY.

Although after drinking large quantities of water the haemolytic power of urine is increased owing no doubt to a change in tonicity, this has no bearing on the phenomenon as ordinarily observed. The resistance series is sufficient to exclude Tonicity as a factor in this haemolytic reaction. In addition there are the surface tension readings out - standing as indicative of a surface active substance being responsible entirely apart from any changes in tonicity which occur.

Ponder has also observed a fact confirmed here that diluted specimens of urine when added to the saline suspension of blood which would tend to make it hypotonic,are, as a rule,non-haemolytic.

He has also noted that the addition of 0.1 cc. of distilled water to 1.0 cc. of the blood suspension will not cause haemolysis,whereas the addition of 0.1 cc. of a haemolytic urine more equal to that of the blood in tonicity, is sufficient to cause haemolysis.

The Reaction of The Urine.

Ponder's results of observations regarding this point in relation to the lytic effect of urine, are summarised by stating that he found the haemolytic action in both alkaline and acid urines. He found that the degree of acidity did not appear to exercise any effect. It has been shown by another observer that solutions acid to P_H 5.1 produce haemolysis, and as Ponder states this has no bearing on the haemolysis of urine (P_H 6 approx.) when such a urine is diluted 1 in 10.

Here again it can be noted that the high degree of correlation between surface tension of urine and its haemolytic activity excludes such a factor as reaction when these specimens were unselected so far as reaction is concerned.

S E C T I O N 6

D I S C U S S I O N

A N D

C O N C L U S I O N S.

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DISCUSSION.

The presence of a haemolytic substance in normal urine and in urine from pathological conditions has been fully shown. The periodicity of the occurrence has been noted. The difficulties of isolating the lytic agent have to some extent been mentioned. As Ponder stated, urine, if evaporated to dryness over a water-bath (a lengthy process during which it is uncertain exactly what changes may occur) and the residue treated with alcohol a haemolytic fluid is obtained.

A litre of urine was evaporated in the above manner. It is not certain, however, if such a residue is dissolved in alcohol how many substances are taken up, or what changes have occurred which may influence the reaction, and this method does not assist in solving the problem as to the nature of the haemolysin.

Again if the bile salts could be extracted from the urine and the urine then tested for the haemolytic reaction, this would be of value in ascertaining to what extent, if any, the bile salt content is responsible for the haemolysis.

For similar reasons the bile salts cannot be removed chemically without the substances used for the extraction having an appreciable effect on the haemolysis, /

haemolysis, if not in themselves producing haemolysis. Filtering the urine is useless; bile salts like other haemolytic substances remain to some extent on the filter, but again to what extent is not certain. (It may be noted here with regard to the unreliability of Oliver's Test in low concentrations which is shown later, that urines filtered give the same degree of opacity in most cases before and after filtration, if any opacity which is present in the urine before filtration is allowed for or more accurately observed in urine just passed which presents no degree of opacity before filtration. Indeed Oliver's test depends on filtering the urines till they are absolutely clear before the addition of the testing solution.).

If urine be shaken up with charcoal according to two observers the haemolytic substance is removed.

This, however, is open to the same fallacies as extracting with alcohol as it is not known what substances are taken up and the isolated substance or substances cannot readily be tested.

To overcome these and other difficulties, in the first place, methods already shown were adopted - the attempt to obtain a percentage haemolysis curve for urine to observe how it compared with the percentage haemolysis curve of other known haemolytic substances - a resistance series for the same reason - and/

and also to find if the surface tension of urine measured by an accurate method bore a relationship to the haemolytic activity - knowing that bile salts were surface acting as also are several of the other well known haemolysins which might conceivably be present in urine.

With regard to the percentage haemolysis curve, it was found that this curve was suggestive of the percentage curve of saponin rather than that of sodium taurocholate.

The resistance series gave no further indication as to the nature of the substance save that the series agreed with the series obtained with the saponin series.

Similarly the changes in the cells during haemolysis observed under the microscope at a temperature of 37°C. only indicate that the changes observed correspond to the changes produced when the common haemolysins are acting on the cells.

The definite relationship between the surface tension of urine and its haemolytic activity is an important observation, showing that the lower the surface tension of urine the greater its haemolytic activity. This observation proves beyond reasonable doubt that the responsible substance is very surface active and it was then necessary to consider substances which were haemolytic and produced at the same time marked alterations in the surface tensions - and also substances which might be reasonably expected

to/

to occur in urine, if not commonly known to do so.

Bile acids and their salts were naturally considered first, as they supply these essentials.

Firstly then the normal bile acid content of urine must be considered. The observer who found this by evaporating an exceedingly large quantity of urine to dryness and then estimating the bile acid content was Dragendorff, and he found this to be not more than 1 in 100,000.

What was then indicated, therefore, was to add sodium taurocholate solution of various concentrations to urine and estimate both the surface tension and the haemolytic activity before and after the additions. This is, however, unsatisfactory because the urine may be in itself haemolytically active, or may, if non-haemolytic, contain substances which retard or accelerate the reaction. With a synthetic urine the same difficulties arise - it is not known to what extent some of the less common constituents of a urine prepared in such a way, - which is at the best not a very reliable substance for comparison - may influence the reaction.

For this reason, therefore, a solution was prepared with the sodium chloride and urea content of normal urine which are after all the main constituents and which are known in themselves not to cause haemolysis or affect the haemolytic phenomenon - a fact shown by the controls.

The surface tension of this fluid was taken by the stalagmometer and is given on the table below.

To this sodium taurocholate was added, made up in concentrations of from 1 in 1,000 to 1 in 100,000.

Tubes were then put up exactly similar to those in which the ordinary urines were examined, i.e. in dilutions of 1.0 cc. to 0.1 cc. of this solution with 0.9 cc. saline (0.85%) as described under "Technique" in Section I, in the case of all the concentrations of sodium taurocholate, and 1 cc. of human blood suspension 0.5% strength was added to each tube and all tubes incubated for several hours at a temperature of 42°C. as before.

Readings were taken every fifteen minutes and the following results were obtained:-

Surface Tension Readings of this "synthetic urine" by the Stalagmometer before and after the addition of various dilutions of Sodium Taurocholate.

The "urine" -	Stalagmometer Reading	21 + 10.
1 in 1000 sod.taurochol. and "urine".	"	40 + 5
1 in 10,000.	"	27 + 10
1 in 20,000.	"	25 + 0
1 in 40,000.	"	24 + 15
1 in 80,000.	"	22 + 12
1 in 100,000.	"	22 + 20,

Haemolytic Activity of this "urine" with the various dilutions of sodium taurocholate solution.

No haemolysis was produced by the "urine" without any addition of sodium taurocholate solution.

	1 in 1000	1 in 10,000	1 in 20,000	1 in 40,000	1 in 80,000.
Minutes.					
5	0.5	0	0	0	0
10	0.5	0	0	0	0
15	0.25	0	0	0	0
30	0.25	0	0	0	0
60	0.1	0	0	0	0
75	-	1.0	0	0	0
90	-	0.75	0	0	0
105	-	0.75	0	0	0
120	-	0.75	0	0	0
Hours.					
3	-	0.75	0	0	0
4	-	0.75	0	0	0
5	-	0.75	0	0	0
6	-	0.5	0	0	0

It will be seen from the table just given, regarding the surface tension readings that there is a marked difference between the 1 in 1000 concentration of sodium taurocholate and the 1 in 100,000 - just what is expected from a surface active substance such as this.

But if the bile acid content is normally near to 1 in 100,000 as Dragendorff states, it will be observed that this does not agree with the suggestion that the bile acids are responsible for the haemolytic reaction. This would mean that to obtain the common variations in surface tension which have been observed in the section on the surface tension of urines, the bile acid content of urine would vary between 1 in 10,000 and 1 in 100,000.

Before considering such a possibility, if the haemolytic activity of this "urine" with additions of sodium taurocholate in concentrations of from 1 in 1000 to 1 in 100,000 be observed from the table, it is found that a very important difference is manifest.

The readings here are entirely in agreement with those which Ponder obtained. While the 1 in 1000 concentration produced very rapid haemolysis of the suspension comparatively (5 minutes) - six hours incubation at 42°C. were required before the 0.5 cc. dilution/

dilution of 1 in 10,000 concentration of sodium taurocholate with 0.5 cc. 0.85% NaCl produced complete haemolysis. In fact from 4 to 6 hours practically no further haemolysis takes place. After 6 hours the readings were rather irregular and difficult to read, but up to 6 hours there was absolutely no difficulty whatsoever, and this is the important time for it has been shown that with many of the urines previously tested in the other experiments, complete haemolysis of 1 cc. suspension was effected at a higher dilution than 0.5 cc. (e.g. 0.25 cc. and 0.1 cc) in a much shorter period than 6 hours.

This would indicate that at the 0.1cc. dilution of ordinary urine which produced complete haemolysis, that the bile acid content of that tube, according to Dragendorff's calculation, would be somewhere about 1 in 100,000 x 9 = 1 in 900,000, which is certainly very much against the suggestion that the bile acids are the haemolytic agents themselves.

Indeed this dilution or even a considerably higher concentration would not indicate surface activity as registered by the stalagmometer, for it is noted that at a concentration of 1 in 100,000 the surface tension is practically the same as with no addition of sodium taurocholate whatsoever.

Summarising, it is obvious, therefore, if Dragendorff's observation is correct, that the surface acting substance responsible for the haemolytic power of/

of urine is more surface active than the bile salts and more actively haemolytic. Alternatively the bile salts must occur in urine to a concentration of from 1 in 10,000 to 1 in 20,000, which is not near the figure which Dragendorff obtained, and it is unlikely that such an error would occur with the large quantity of urine taken for Dragendorff's experiments.

Considering the next substances which are as haemolytic or more haemolytic than the bile salts, and which are more surface active in the same concentrations, and which might conceivably be present in urine, it was thought that the soaps might have a bearing on the problem, for example sodium oleate, and potassium oleate, for the reason that these are very surface active, though results of estimation of surface tension are exceedingly variable; that they are haemolytic, and they might reasonably be expected to be present in minute quantities in urine because soaps occur in the blood stream and are diffusible.

Matthews states that bile contains small amounts of sodium salts of various fatty acids (myristic, palmitic, stearic) among which sodium oleate may be specially mentioned. The presence of these soaps affects the ease of precipitation of the bile salts by neutral salts, the presence of sodium oleate particularly interfering with salting out.

The problem which arises here is that it is not known/

known in what concentrations these might occur.

Potassium and sodium oleate being the most actively haemolytic of the series, were accordingly selected for the experiments.

The difficulty in reading haemolysis in the case of soaps is great. In fact at 42°C. which is the temperature at which all the other experiments were carried out, readings were impossible after half an hour, owing to the opalescence which formed due to the temperature, although the purest specimens were obtained.

The concentrations prepared were the same as those used with sodium taurocholate.

Sodium oleate was found to produce quite as rapid haemolysis of the suspension as sodium taurocholate, but by the time the 1 in 1000 concentration had caused complete haemolysis, the lower concentrations were impossible to read on account of opalescence.

Regarding next the surface tensions of these soap solutions it was found in the case of potassium oleate the surface tension of the 1 in 1000 to 1 in 100,000 was even lower than the corresponding concentrations of sodium taurocholate - therefore even more surface active, but with sodium oleate this was not found to be the case.

Ponder in a paper on the haemolytic action of soaps/

soaps has stated that in estimations of surface tension of sodium oleate in saline solutions concordant results are very difficult to obtain, and that there is no definite value for $d\sigma/dc$.

In suggesting that soaps may quite possibly be present in urine then, and the cause either wholly or in part for the haemolytic activity, that present would require to be a constant type or there would require to be a tendency for other factors already present in the urine to preserve this. In any case, it might be, with very low concentrations, which could only be expected to be present in urine, that the difficulties in reading due entirely to the opalescence obtained on heating would not be so great or if soaps were present in urine other factors might prevent to some extent such an occurrence.

For that matter the bile salts themselves show to some extent this opalescence on heating, though not to the same extent as soaps, but this degree of opalescence is not seen in urines when heated to bear a relationship to the haemolytic activity; but on the other hand many other substances may cause opacity in urine which is heated which would mask such a parallelism even if present.

Ponder again emphasizes the necessity of obtaining pure specimens because he found that impure samples gave rise to serious errors; such impurities being/

being alkali or even saponin. He has also pointed out that soaps are highly unsatisfactory substances and when quantitative data as to their haemolytic activity are required; being semi-colloids they exhibit phenomena characteristic of their class; their physical properties show a great tendency to alteration from time to time; the time factor having to be taken into account in all determinations of their surface tension and even their haemolytic activity.

The time factor in comparing the haemolytic activity of soaps with that of urine was a drawback and again the low temperatures which Ponder used for his complete results were naturally unsuitable for comparison with the haemolytic activity of urine if the comparison was to be accurate.

If one of the soaps were the responsible factor in the haemolytic action of urine, it would, as stated, require to be a very constant specimen and one which produced a fairly constant alteration in surface tension. It is conceivable that such could occur although the surface tension readings of sodium oleate in saline are not constant with the stalagmometer.

With regard to the reading of the degree of haemolysis in the tubes which was impossible on account of the opalescence which invariably occurs on heating the soaps, the intact cells could be centrifuged off and the colour of the supernatent fluid compared with standard/

standard tubes, but this technique would not be accurate enough for such observations.

As previously stated with Oliver's testing solution added to the solution of sodium chloride and urea used in place of urine, plus varying concentrations of sodium taurocholate from 1 in 500 to 1 in 100,000, it was found that no precipitation occurred at a concentration of 1 in 1000 sodium taurocholate. This is certainly most suggestive that this precipitate obtained when Oliver's solution is added to urine, taking the bile acid content of urine to be near 1 in 100,000, is not due to bile acids and that Oliver's test is unreliable in such low concentrations, although certainly the precipitate obtained with the 1 in 500 concentration was very definite. Oliver's test depends upon the precipitation of peptone in acid solution and it is quite possible that some other surface active substance may give this precipitation. As shown from the graphs in Section I Oliver's test readings do not run parallel to the haemolytic activity in either normal urines or those from unselected pathological conditions.

If the precipitate obtained by Oliver's test in urine is not due to bile acids of course bile acids might still be the responsible agent in haemolysis for that matter, had not other factors been shown to be against this.

In support of this we have seen that in filtering urines/

urines the same degree of opacity is often obtained by the addition of Oliver's testing solution before and after filtration, despite the fact that we know bile salts like other haemolysins to remain to some extent on the filter.

It is agreed that in concentrations of sodium taurocholate 1 in 500 the reaction is due to bile acids, but this again would indicate that the bile acid content of urine - taking the average degree of opacity of precipitate from the urines tested and comparing it with that obtained with the opacity given with 1 in 500 to 1 in 750 sodium taurocholate that the bile acid content of normal urine was somewhere about 1 in 750, which is almost certainly not the case.

Whatever might be the constituent responsible for the precipitation obtained with Oliver's test added to filtered urine has no bearing on this problem since the precipitate was not found to agree with the haemolytic activity, and indeed the soaps do not give this opacity with Oliver's test in concentrations of from 1 in 1000 to 1 in 100,000; and even if any of the soaps were the cause of the haemolytic reaction of urine this would be quite in agreement with the findings as regards Oliver's test obtained in Section I.

It must be remembered that the bile salts themselves are closely associated with the soaps; there is practically/

practically no method of definitely distinguishing the two in an investigation such as this.

Ponder found a relationship between Oliver's test readings and the haemolytic activity of the urines, but he incubated his tubes after the Oliver's testing solution had been added, and this may account to some extent for the difference obtained - for example with the precipitate already present plus an opalescence due it is suggested from a soap, may have agreed with the haemolytic acitivity - similarly an opalescence occurs though to a less extent on heating the bile salts.

Summarising, the suggestion which has been put forward that bile acids and traces of their salts are the lytic agents of urine. We have the graphs in Section I, but those readings obtained with Oliver's Test cannot be taken into account as the precipitate obtained is possibly not due to Bile acids, and although Ponder found a parallelism such is not obtained here, but since we have shown that Oliver's test is unreliable in low concentrations, such a method of determining any relationship between the haemolytic action of urine and the bile acid content is suggested as being of no value.

In the urines of certain pathological conditions containing bile either obviously by naked eye or nitric acid test, the extra degree of lowered surface tension/

tension is apparent, but these urines are not necessarily even as haemolytic as urines in which no bile is present, i.e. urines with no abnormal constituents. Examples of this type of urine are shown in the Section on surface tension. In such urines of course the precipitate obtained in Oliver's test is marked, and it is possible here that the concentration may easily be 1 in 500 or more.

In some of the notes with the graphs and elsewhere that arguments are stated, such as that two specimens of urine (the same or different urines) may give the same degree of opacity with Oliver's test and yet vary in haemolytic activity, and it will be remembered that these notes were inserted before the low concentrations of sodium taurocholate solution was tested by Oliver's Test, the latter being taken as reliable for such concentrations at this time.

CONCLUSIONS.

(1) We conclude, therefore, that urine contains a haemolytic substance or substances and that all urines exhibit this haemolytic activity if observed over several days. This haemolytic power varies, however, to a marked extent and indeed may be absent for several days.

(2) From the examination of urines from unselected pathological conditions we conclude that this haemolysin is present in the urines of pathological states to much the same extent as in urine from healthy individuals. In no particular disease have we found any constant variation from the normal. There are certain indications that the absence of this haemolytic power may be associated with some cases of pernicious anaemia, but too few cases of this disease were available to allow any definite statement to be made regarding this point.

(3) In finding that this haemolytic activity is present in normal urines we differ from the observations by McKee; the reason for differing lying in the fact of defects in technique by this observer. We have considered at length this/

this matter of technique and have shown that it is necessary to incubate for longer periods than have been hitherto employed, if accurate results are to be obtained.

Before a urine is pronounced non-haemolytic several examinations on different days are required and abnormal constituents such as albumen, blood, and pus must be excluded.

(4) We have been unable to observe that any drug used in treatment has any bearing on the haemolytic activity: similarly age appears to bear no relationship to the haemolytic power.

(5) Following the suggestion of Ponder we have attempted to obtain a parallelism between the bile acid content of urine as estimated by Oliver's Bile Acid Test and the haemolytic activity, but we have not found that such a parallelism exists, the test being positive at certain times when the urine was non-haemolytic, and negative when the urine was very actively haemolytic, and in general not varying with the haemolytic power.

We have shown, however, that Oliver's Bile Acid test is evidently not reliable in dilutions greater than 1 in 1000, and that it is therefore not a suitable test for bile acids to the extent/

extent to which they occur in normal urine. We have been unable to discover a substance which will yield the precipitate which occurs in normal urine when the above test is applied, but on general principles we are inclined to regard it as some surface-acting substance appearing in high dilution. Even if this be so we have not been able to obtain evidence that the presence of this hypothetical substance confers a haemolytic property to the urine.

(6) Specific Gravity and p H of urine appear to have no bearing on the problem and tonicity has no bearing on the reaction as ordinarily observed.

(7) In endeavouring to discover the nature of the haemolytic substance which is, according to Ponder, heat-stable and extractible by alcohol, we have attempted to relate it to other known haemolysins by observing characteristics of the haemolytic reaction which depends upon its presence.

The percentage haemolysis curve for the lytic agent in urine is somewhat similar to that of Saponin and certain of the Soaps and although it is impossible to be dogmatic owing to technical difficulties we are inclined to think/

think that it is dissimilar to the percentage haemolysis curve of the bile salts. The curve exhibits a skewness which is suggestive of the saponin type rather than the bile salt type, but we cannot emphasize this point unduly.

We have obtained one piece of evidence that carries weight in the argument, that the lysin is not of the bile salt type, that being that the addition of serum to the haemolytic system after haemolysis has partially occurred does not result in acceleration of the reaction as found with the bile salts as lysins, but an inhibition as occurs in the case of saponin and certain soaps.

(8) Testing the red cells of different animals against haemolytic urine we find that they may be placed in the following order:-

Rabbit, man, ox, sheep, from least resistant to most resistant, and this resistance series, known as Ryvosh's series, is common to simple haemolysins of the saponin type. The appearance of this order does not exclude the possibility of the bile salts being the haemolysin but excludes tonicity effects. Observations have shown us the necessity of testing for this series with dilute suspensions whose cell content is comparable, for if concentrated/

concentrated suspensions are employed and totally different series may be obtained owing to fallacies in technique.

(9) Following the suggestion that the haemolytic substance is a surface active substance of unknown nature we have estimated by means of the Stalagmometer a large number of urines and have compared the results with the haemolytic activity. By this means a high degree of correlation between surface tensions of urines and their haemolytic activity has been obtained, the most actively haemolytic urines possessing the lowest surface tension and vice versa - the excursion of variation in the surface tension of urines being from 75 to 50 dynes / cm.

In connection with this we have noted the interesting fact that where bile is present as a constituent of urine to the extent it occurs in disease, e.g. catarrhal jaundice - the surface tension is naturally low and the haemolytic activity apparent, but it is not so great as some urines which have a comparatively low surface tension but do not contain bile, i.e. the extra degree of lowered surface tension produced by the bile present does not produce a corresponding added degree of haemolytic power.

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This is a further argument to the hypothesis that Bile Salts are not the substances which confer this haemolytic property on urine.

(10) Investigating the subject from another point of view by estimating surface tensions of solutions of bile salts in varying concentrations, we conclude that the order of dilution which gives a surface tension comparable to that observed in haemolytic urines is from 1 in 10,000 to 1 in 100,000, but the order of dilution which gives haemolysis comparable to that obtained with urines is 1 in 10,000 to 1 in 20,000.

The discrepancy between the two orders is so great, it seems highly improbable that the haemolytic power is due to the bile salts. An objection to this may be that urine may contain substances which accelerate the haemolytic action of the bile salts, but this is not the case, for the effect of urine on bile salt haemolysis after partial haemolysis has been effected is an inhibitory one, as previously stated, which makes the above argument stronger.

(11) Since some soaps are known to be actively haemolytic, surface active, and appear in the blood stream, and that they are diffusible, we have examined the possibility of the responsible factor/

factor being a soap. It could not be a palmitate or a stearate for these, according to Ponder, are comparatively non-haemolytic. Confining our attentions then to oleates we have found that the surface tensions of these are notoriously difficult to determine. This has been shown by several observers to vary in an inconstant manner with dilution.

With potassium oleate we found the surface tension to be even lower than corresponding concentrations of sodium taurocholate but not so in the case of sodium oleate. The haemolytic activity of varying concentrations of these soaps, however, is exceedingly difficult to determine as soap becomes opalescent at the temperature which was required for comparison here.

Judging from the figures by Ponder this concentration is of the order which might be expected to produce lysis and it is possible if we take into consideration the point above mentioned that the haemolysis might be potassium oleate.

It is difficult to see however why the opalescence which occurs when this concentration, (1 - 100,000) is used, that the opalescence does not necessarily occur when haemolytic urines are heated, and it is difficult to explain the/

the constancy of the surface tension readings in view of the fact that the oleates give no steady values for surface tension by the Stalagmometer method. The form of the percentage haemolysis curve, the failure of addition of serum to accelerate the reaction when the cells are partially haemolysed, the order of resistance series, are all in keeping with the suggestion that the lysin may be an oleate. Conclusive evidence is not forthcoming owing to the fact that whether we measure haemolytic activity or surface tension of oleates the results are very difficult to obtain and very variable.

(12) Drinking large quantities of water produces an increase in haemolytic activity, due probably to the fact of a lowering of tonicity of the urine, but this has little if any bearing on the phenomenon as ordinarily observed. Tonicity effects have been excluded by the resistance series and more definitely by the relationship between surface tensions and haemolytic activity.

In conclusion we believe that there is evidence that the haemolysin contained in urine is not one of the bile salts but is a very surface active substance of the saponin-soap class; it is possible that it is an oleate.

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