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215 MEDICAL ARTS BUILDING
WINNIPEG

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Faculty of Medicine
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Dear Dr. Mathers;

Dr. F.D. White handed me Dr. Coke's thesis which has been submitted for consideration for the Prowse Prize. I have read the report with considerable interest and would offer the following observations.

1. The report would indicate that a large amount of careful work was done but one must regret the absence of detailed protocols. The figures contained in the thesis are ingenious but serve to give only a bare summary of results rather than the detail which is so necessary to proper evaluation.

2. The body of the report is very sketchy. No effort is made to give any historical background and the reader would have to read all the references given in extenso to form a proper basis of judgement.

3. Dr. Coke has failed to make a critical study or interpretation of the limitations of a lone study of urinary excretion of Vitamin C. A consideration of the known facts of its metabolism and the information which is still required would have given a good background for the investigation.

4. Dr. Coke appears to have made good use of the wealth of material available in a sanatorium for study and to have made a most industrious study. The shortcomings noted above may be attributed I think to lack of experience and training in reporting an original investigation.

I have personnal reason to know Dr. Coke's real ability and feel that he is to be commended for his work;my chief criticism is that the report is inadequate to the study. I have made no specific recommendation re the award as I feel that this is a matter for discussion among the several assessors.

Yours very truly

Our investigation began following the reports of Abbassy, Hill and Harris,[1] Heiss and Martin,[2] and Bullowa and others[3] of vitamin C deficiencies in tuberculosis, and the greater part of our work has been in this connection. What is described below, however, is a series of incidental experiments performed in the course of this work which happened to explain a number of obscurities.

(1) Abbassy, M.A., Hill, N.G., Harris, L.J.,
Lancet December 12, 1936. p. 1413.

(2) Heiss, F.H., Martin, G.J.
Proc. Soc. Exp. Biol. Med. 34 . 642 . 1936.

(3) Bullowa, J.G.M., et al.
Proc. Soc. Exp. Biol. Med. 34 . 1 . 1936.

Technique.

The technique as described by Harris and Ray[4] for the determination of ascorbic acid in urine with 2 6 dichlorophenol indophenol was used. All tittrations were done by the writer. It was found convenient in collecting specimens to send test tubes containing 1 c.c. of glacial ascetic acid to the ward. The nurses added 9 c.c. from the specimen in question and marked the total quantity and the time on the tube. Results obtained in the summer of 1937 were later entirely disregarded because it was found that rapid deterioration of ascorbic acid had been taking place in spite of the immediate addition of preservative. It was subsequently shown that deterioration could be limited to less than 3% in 12 hours when the acidified specimens were kept in a refrigerator at a temperature of 45 degrees F.

The importance of keeping specimens cold has been mentioned by Bullowa,[3] Everson and Daniels,[5] and Hawley.[6] Patients had all been one or more months in sanatorium so that dietary inequalities were disregarded. Of the first twenty unselected cases of tuberculosis the twenty-four hour excretion was found to be below the normal standard[7] in thirteen patients, which is in accordance with the report of Hurford.[8] However, as in the case of Hurford, we were unable satisfactorily to correlate the degree of apparent deficiency with the extent and activity of the tuberculosis.

(4) Harris, L.J., and Ray, S.M.
Lancet Jan.12. 71. 1935.

(5) Everson, G.J., Daniels, A.L.
Journal of Nutrition 12. 15. 1936.

(6) Hawley, E.E.
Journal of Pediatrics 12. 381. 1936.

(7) Abbassy, M.A., Harris, L.J., Ray, S.M., Marrack, J.R.
Lancet Dec. 31. 1935. p. 1399.

(8) Hurford, J.V.

Brit. J. Tuberculosis 22 121 April, 1938.

Unable, then, to attribute our results entirely to the presence of disease, a search was undertaken for other causes of variation.

Abbassy, Harris, Ray and Marrack[7] described a fall in ascorbic acid excretion under controlled conditions during fever due to a cold, and attributed the result to pyrexia.

Abbassy, Harris and Hill[9] also observed a diminished rate of excretion during pyrexia in cases of osteomyelitis and Harris apparently believes that pyrexia causes an increased utilization of ascorbic acid.

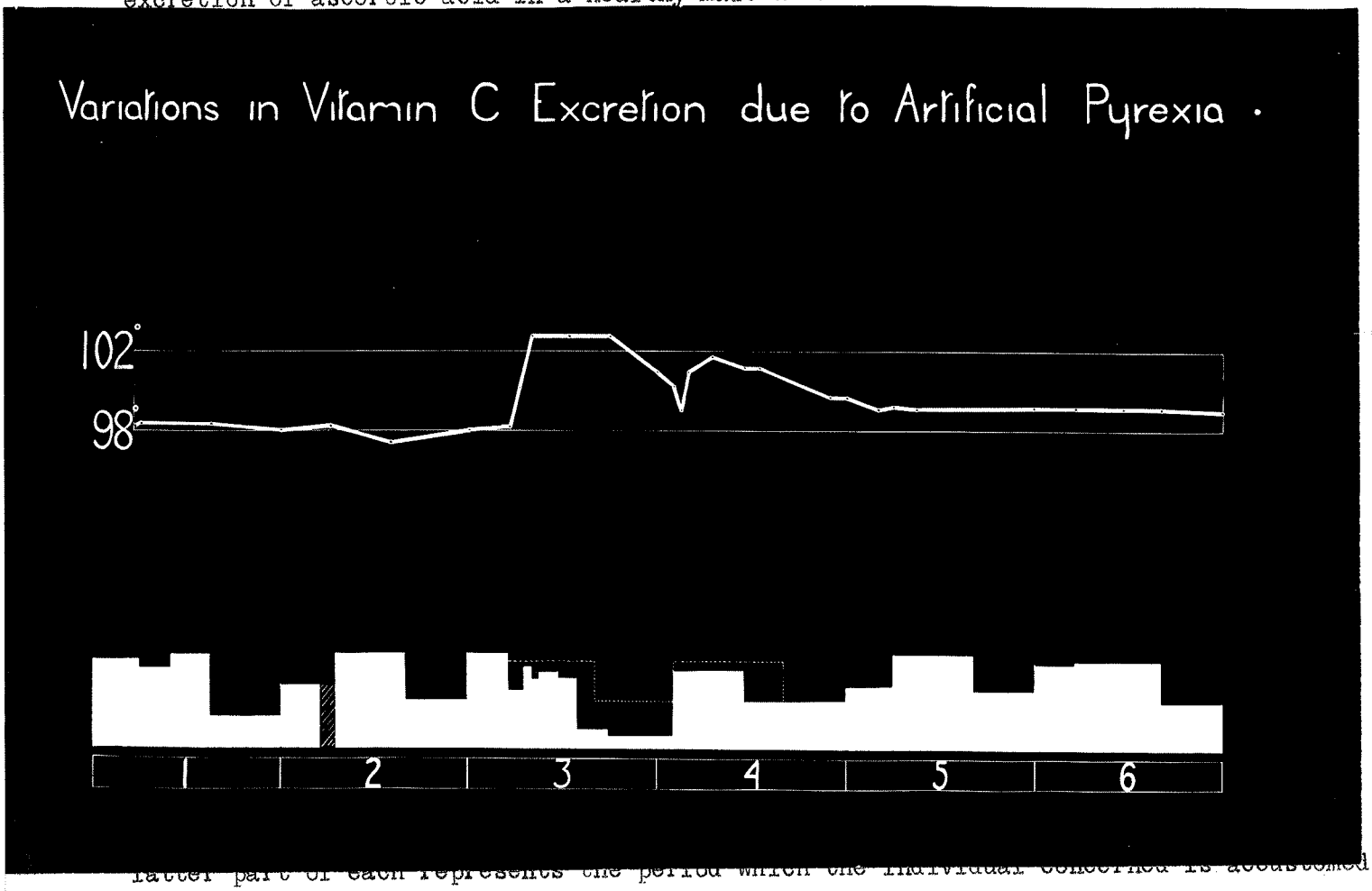
Hurford[8] gave statistical evidence to show that pyrexia in tuberculosis did not affect the rate of excretion.

However, in none of these cases was the element of toxemia entirely eliminated.

(9) Abbassy, M.A., Harris, L.J.

Lancet, July 24, 1937. p.177.

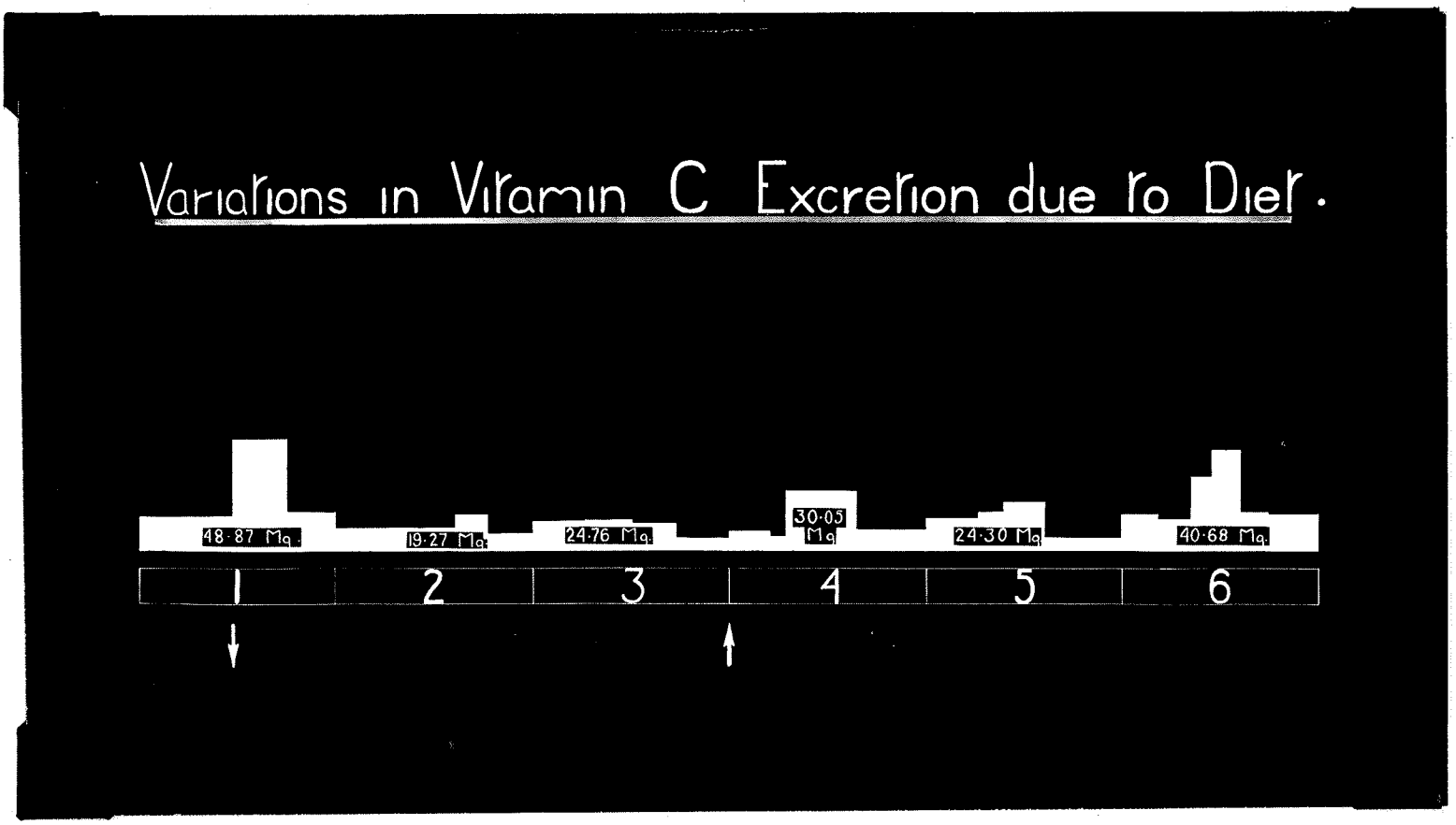
In the following experiment the effect of artificial pyrexia on the excretion of ascorbic acid in a healthy male adult was measured. Diet and



latter part of each represents the period which the individual concerned is accustomed to recognise as night. On the day marked "3" an intravenous injection of 100,000,000 dead typhoid organisms was given, with the resulting rise in temperature. The dotted lines show the approximate excretion anticipated apart from any interference. Generally it is seen that the rate of excretion falls from 26 mg. on each of the first two days, to 19 mg. on the third and fourth, and returns to 27 mg. on the fifth and sixth.

It seems therefore reasonable to make allowance for a decreased rate of excretion in pyrexia.

The second experiment was on the effect of cessation of intake of vitamin in food on a healthy individual. The results are shown in the chart.



Days begin at 8.00 a.m. so that the latter part of each represents the period of sleep. A period of six days is represented. The arrows indicate the cessation and resumption of eating. Usual activities were studiously carried on.

The fall in the rate of excretion is evident, but more striking is that during starvation the excretion of ascorbic acid continues to be irregular. We had previously thought that the relatively large amount of ascorbic acid excreted in the daytime corresponded to and resulted from the intake of vitamin in food, but it was observed that the daytime excretion during starvation was still considerably higher than that seen during the night. It was also noticed that the excretion on the second day of starvation was appreciably higher than on the first.

It was concluded that cessation of intake caused a moderate decrease in output and that further unknown factors were in operation.

Little reference has been made in the literature to the effect of fluid intake and diuresis on the rate of excretion of ascorbic acid.

Everson and Daniels[10] observed an increase in the ascorbic acid excretion of a child given aspirin and fluids for a cold.

Abbassy[11] reported a diuretic action of ascorbic acid. Under carefully controlled conditions of fluid intake, he was able to show that there was a constant increase in fluid output occurring at the time of saturation and the appearance of large quantities of ascorbic acid in the urine.

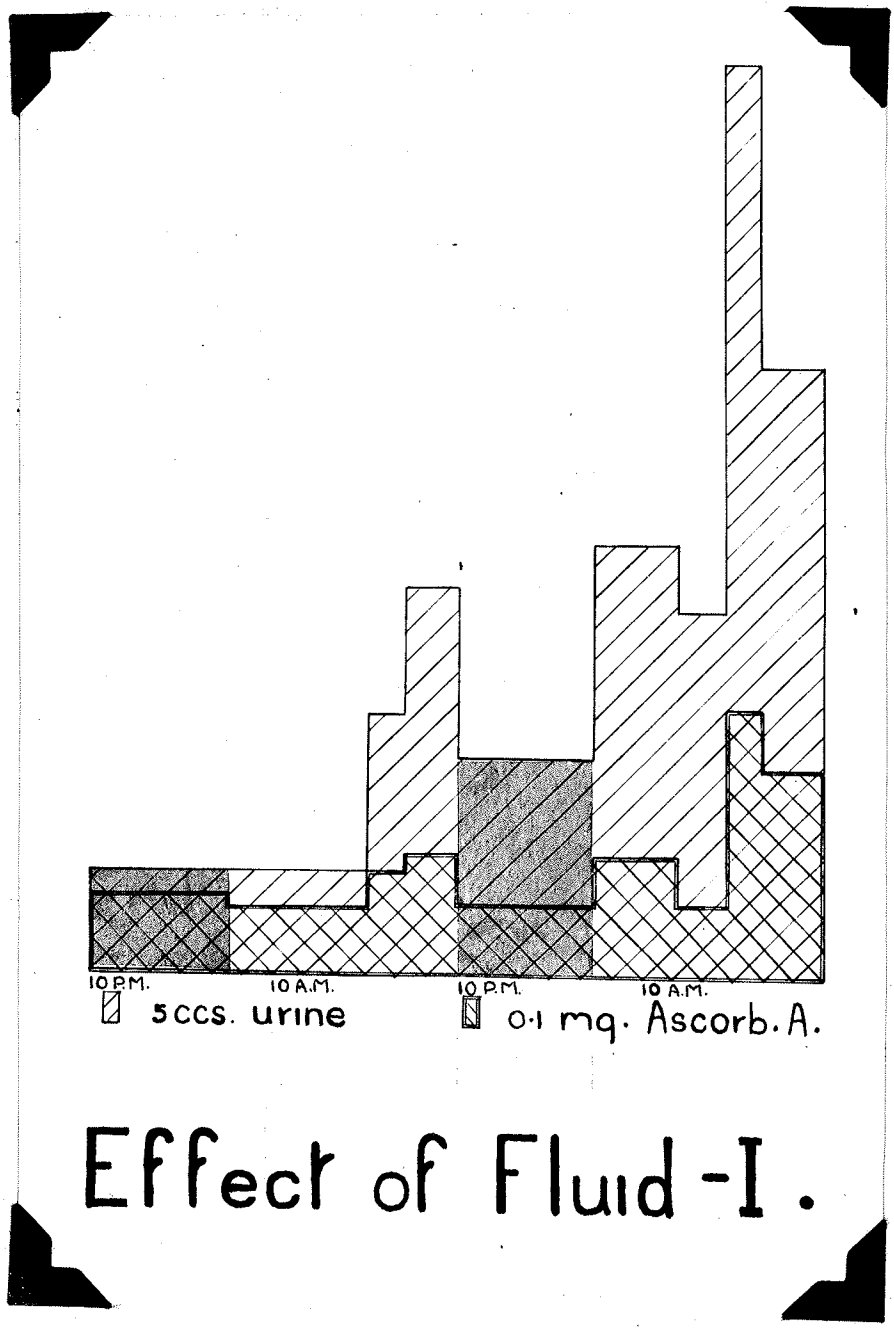
(10) Everson, G.J., Daniels, A.L.

Proc. Soc. Exp. Biol. Med. 35. 20. 1936.

(11) Abbassy, M.A.

Biochem. Jour. 31 No.2 1937.

In the chart marked "Effect of Fluid I," the subject was a young male patient with tuberculosis of the spine.

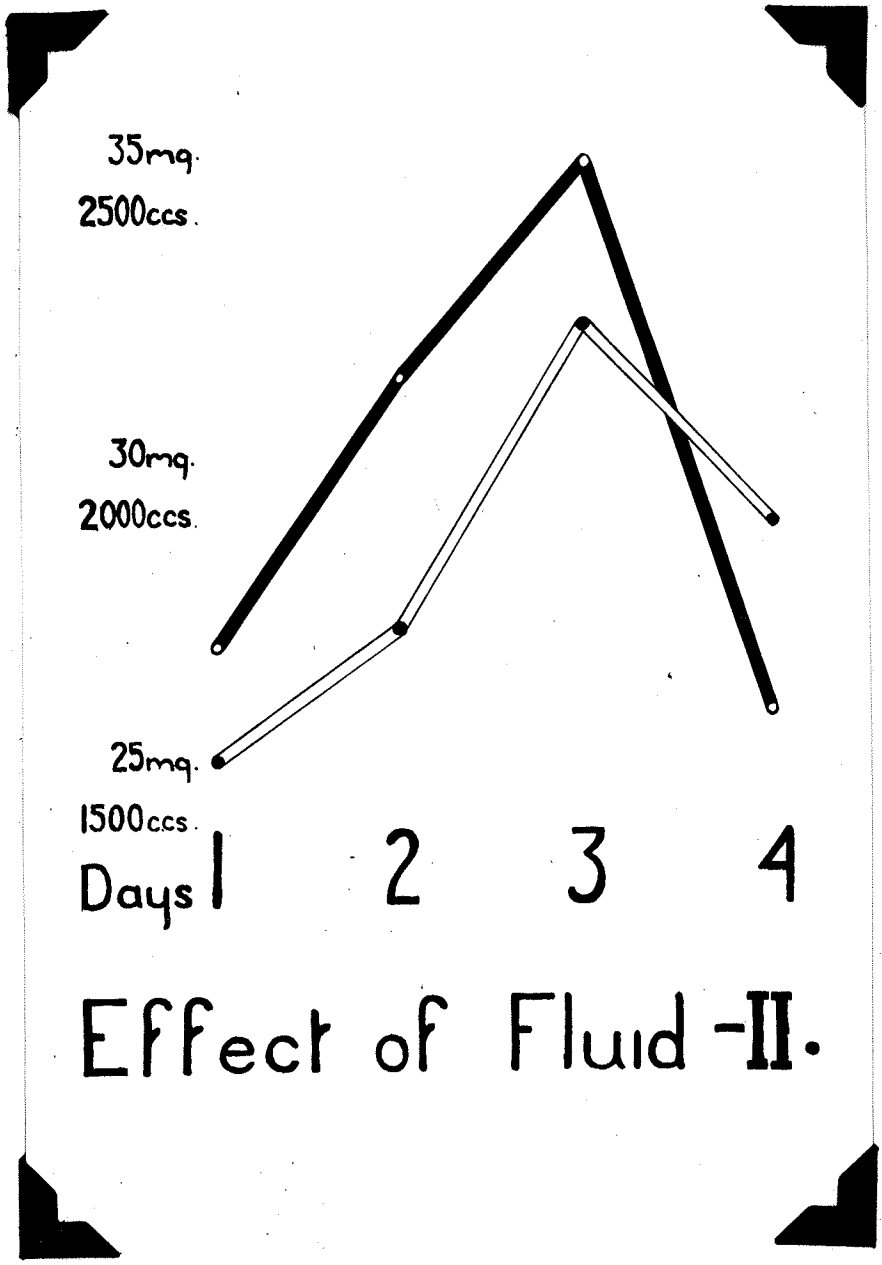


Effect of Fluid - I.

A period of two days is represented, the area shaded corresponding to the period of sleep. Foods containing much Vitamin C were withheld. The parallelism between urinary output and ascorbic acid excretion is clearly seen. It was found that for the first twenty-four hours the excretion of ascorbic acid was 7 mg. and for the second twenty-four hours 10 mg. On the second day the urine output was doubled. It seemed, therefore, that a definite relationship between the quantity of urine and the amount of ascorbic acid excreted in a given time could be expected.

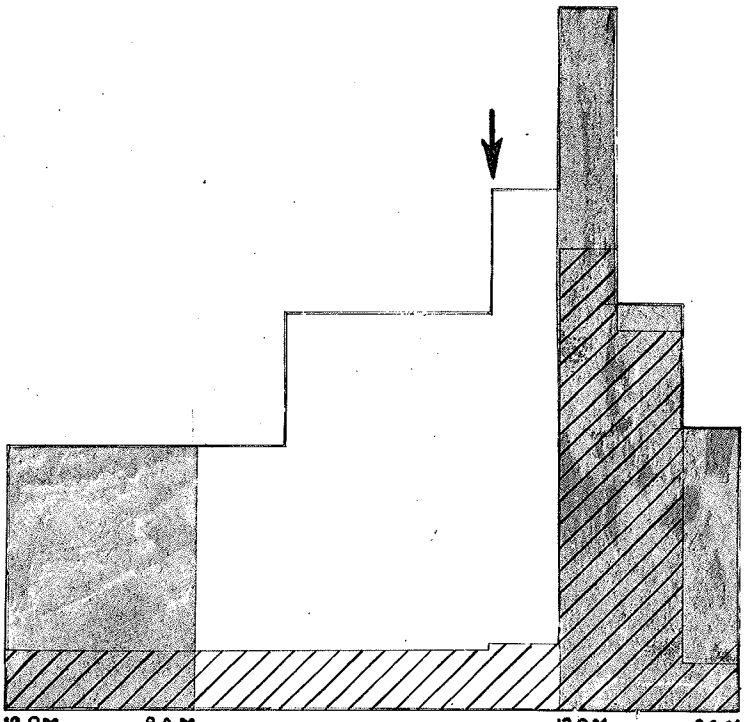
In our second experiment, in which variations of excretion were found during a period in which no food was taken, it was also found that the quantity of urine showed corresponding variations. Furthermore, in the experiment on pyrexia, the fall in output of urine appeared to be sufficient to explain the diminished excretion of ascorbic acid.

It remained to produce changes in the twenty-four hour curve and the twenty-four hour total by alterations of fluid intake. Experiment II on the effect of fluid shows changes in a normal person. Vitamin intake was kept constant.



In this chart the solid line represents the excretion of ascorbic acid, and the plain line the volume of urine. It is seen that with increasing diuresis, there is a corresponding increase in the amount of ascorbic acid excreted. The fall in the ascorbic acid output on the fourth day is greater than that which might have been expected and suggests the possibility of a "washing out."

The last experiment was devised to demonstrate the possibility of reversing the ordinary twenty-four curve by controlling fluid intake. It is complicated by the effect of food, but it is seen that the afternoon rise was delayed so that the greatest concentration of ascorbic acid appeared in the urine after midnight. The result is more significant when this curve is compared with a large series of ordinary curves which are extraordinarily regular.



Effect of Fluid No III.

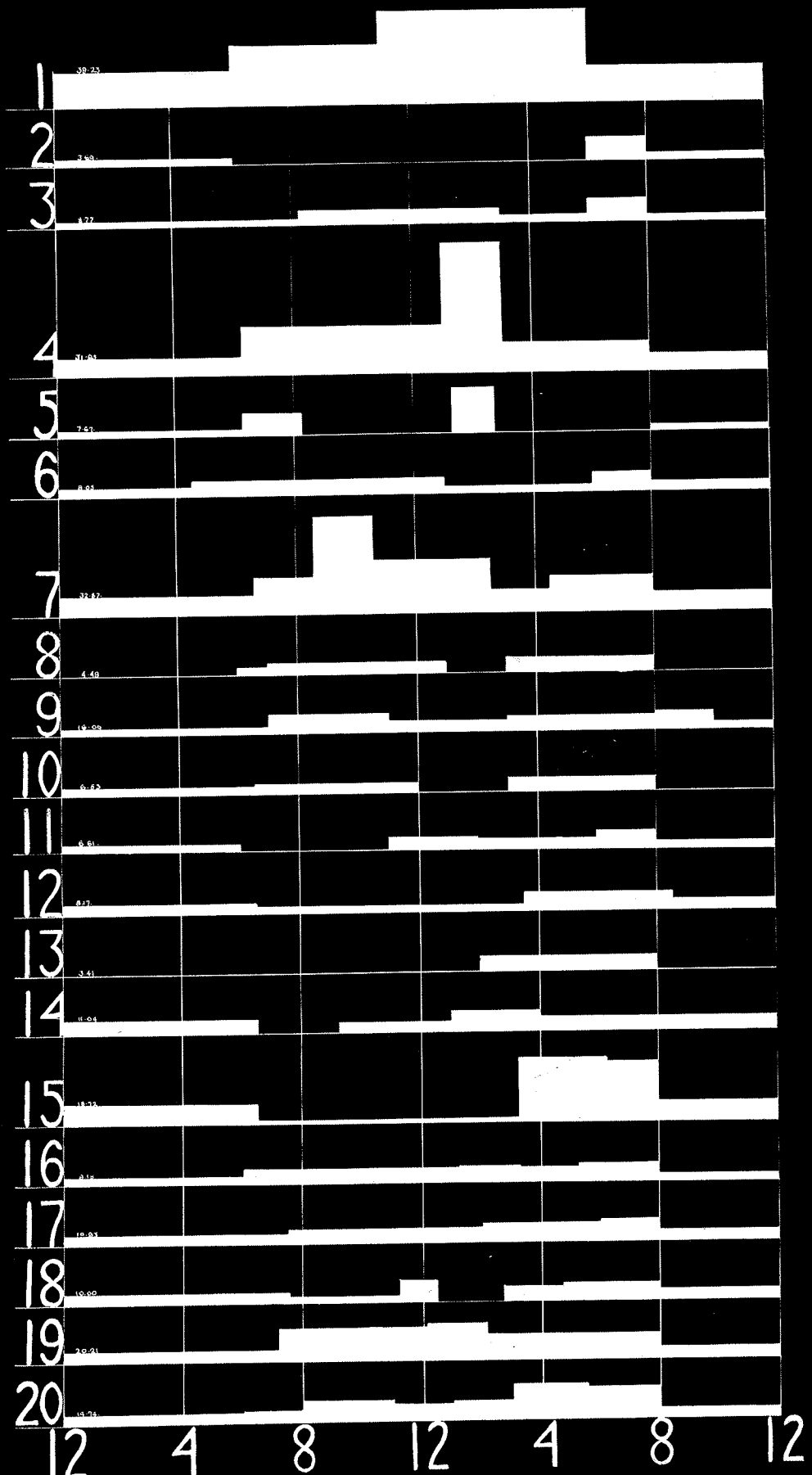
APPENDIX.

The chart marked Excretion of Vitamin C in Twenty Cases of Tb. is an analysis of the diurnal variations in a group of female patients. Disease of different types including minimal and far advanced pulmonary cases are represented.

The cases are numbered 1-20 and the vertical lines represent time beginning at 12 o'clock p.m. (so that the middle one is 12 o'clock noon). The "daytime" excretion is seen to be greatly in excess of that at night and the maximum rate of excretion is invariably found during the day.

It has been suggested that apparent diminution in quantity of ascorbic acid might be the result of retention of urine within the bladder. The results of emptying the bladder intermediately during the night do not, however, support such an explanation. Control of vitamin intake in food and variations in temperature and hydration were not taken into account in these tests so that their further consideration would not seem to be justifiable.

Excretion of Vitamin C in Twenty Cases of Tb.

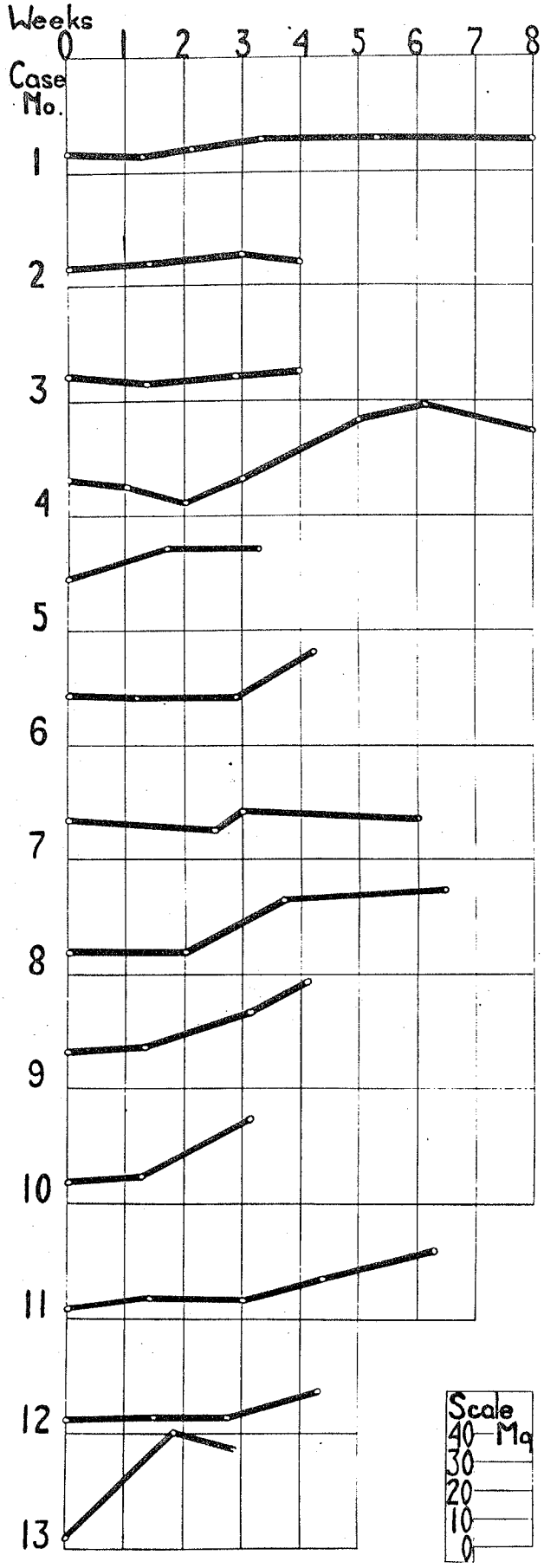


The Chart marked "Saturation Curves" shows the effects of the daily administration of 25 mg. of synthetic Ascorbic Acid (E.R. Squibb) in addition to that contained in sanatorium diet.

A rate of excretion of more than 20 mg. per day is referred to as saturation.

Each point represents a twenty-four hour total. The twelfth patient died during the period of observation. The first three have died subsequently. This type of study would seem to be of greater value but in practise is so laborious that it could scarcely be recommended. From a theoretical view the same objections hold and one wonders whether a large intravenous injection of fluid might not have liberated a quantity of ascorbic acid in some of the so-called "unsaturated" cases.

Saturation Curves



Far adv. pul. Tb., entero-colitis and persistant high fever, failed to saturate in eight weeks.

Far adv. active pul. Tb. and entero-colitis, and doing poorly, failed to saturate in four weeks.

Far adv. active pul. Tb. with entero-colitis and breathlessness, failed to saturate in four weeks.

Very far adv. pul. Tb. making rapid improvement with pneumothorax, saturated in five weeks.

Far adv. chronic pul. Tb. and entero-colitis, showing recent improvement, saturated in ten days.

Far adv. quiescent pul. Tb., saturated in four weeks.

Far adv. pul. disease with peritonitis and cervical adenitis, failed to saturate in six weeks.

Afebrile uncomplicated peritonitis saturated within four weeks.

Uncomplicated peritonitis saturated in three weeks.

Pott's disease with an active psoas abscess, saturated in three weeks.

Severe lupus vulgaris in a child, saturated in seven weeks.

Four year old child with Pott's disease died of meningitis in the sixth week.

Four year old child with tuberculous knee, saturated within two weeks.