THE FATE OF EXTRACORPUSCULAR CIRCULATING HAEMOGLOBIN

BY

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At the present time, when so much work is being done on blood transfusion and blood substitutes, the following observations may be of interest in throwing some light on the fate of extracorporeal circulating haemoglobin. This is especially so since, though the biological relationship of haemoglobin to bilirubin and its subsequent excretion as bile pigment have been established, the relationship between haemoglobin and haematin in normal and pathological conditions in man is less well understood.

I. INTRAVENOUS INJECTIONS OF HAEMOGLOBIN

Duesberg (1934), in an attempt to explain the significance of haematin, as revealed by Schumm's test, in the serum of cases of pernicious anaemia, injected haemoglobin as well as haematin solutions intravenously into man. Amounts of haemoglobin varying from 2 to 6 grammes were injected intravenously. A rise in bilirubin was noted within thirty minutes and the maximum concentration was reached in two to three hours; bilirubin values returned to a normal level after five to eight hours in patients with healthy livers; where the liver was diseased it took eight to twelve hours to return to the original level.

After the injection of 3 to 6 grammes of haemoglobin intravenously Schumm's test was negative even when serum was examined through a depth of 4 cm., except in patients with certain diseases of the liver. Out of ninety-eight patients receiving haemoglobin injections there was only one whose serum gave a positive Schumm reaction in the absence of demonstrable liver disease: this patient had a high blood pressure with transient cerebral attacks. Duesberg (1934) concluded that the occurrence of haematinæmia was dependent on hepatic function, and that in the absence of parenchymatous degeneration of the liver circulating haemoglobin did not give rise to haematinæmia.

As these experimental results were not in conformity with certain recent biochemical findings in the haemoglobinurias and haemolytic anaemias it was decided to repeat Duesberg's experiments, injecting larger quantities of haemoglobin and making quantitative determinations of the resulting haemoglobinaemia, as well as serial van den Bergh and Schumm tests.

Technique Adopted

Quantitative Haemoglobin Determination.—To determine the intensity of the haemoglobinaemia a method was used similar to that of Bloem (1933), in which the minimum concentration at which the α band just disappears from standard solutions of haemoglobin was estimated; to determine the concentration of haemoglobin this value was multiplied by a dilution factor ascertained for the particular specimen of plasma under investigation. The method has been described in detail by Fairley and

Bromfield (1933) and calls for no further comment, except to point out that the plasma haemoglobin must exceed a concentration of 0.12%, or 16.1 mg. per 100 c.cm., before it is significant of haemoglobinaemia, and 25 mg. where no special precautions are adopted to reduce plasmolysis to a minimum. Quantities of haemoglobin below these values may be caused by plasmolysis and cannot be regarded as significant.

Bilirubin Estimations.—Owing to the presence of haemoglobinaemia the original van den Bergh technique was followed by precipitating the plasma proteins with alcohol before adding the diazo reagent. As pointed out by Harrison (1930), this has the advantage over the modified technique of Thannhauser and Andersen of removing the haemoglobin before the addition of the diazo reagent and so avoiding the formation of acid haematin, which makes it difficult to match the standard. It has the possible disadvantage that an unknown quantity of bilirubin is precipitated with the albumin, and this may be the reason why the bilirubin values are not higher in this series of cases. This is suggested by the fact that Pedersen and Waldenström (1937) found on the ultracentrifuge that both forms of bilirubin—i.e., that which couples directly with diazo reagent and that which reacts only indirectly after the addition of alcohol—are bound to serum albumin. Bensley (1934), on the other hand, states that there is fairly general acceptance that loss of bilirubin due to occlusion in the protein precipitate does not occur or is negligible when there is no "direct" bilirubin in the blood, and that where only "indirect" bilirubin is present no significant differences are found between values obtained by the original van den Bergh technique and those obtained by the method of Thannhauser and Andersen.

Schumm's Test.—This test, first introduced by Schumm (1912a, 1912b), has been widely used in clinical medicine to demonstrate haematin in plasma. A considerable literature on haematinæmia has developed on the basis of this test, but recent work done by me indicates that in man haematin does not exist free in human plasma, as it combines with serum albumin to form methaemalbumin. The technique used was as follows:

A given volume of plasma was covered with a layer of ether; one-tenth the volume of concentrated ammonium sulphide (analar) was then run in with a pipette and subsequently mixed by shaking. A positive reaction is indicated by the appearance of a haemochromogen with a sharply defined α band at 558 m μ in a depth up to 4 cm. of plasma. The presence of much haemoglobin adds to the difficulty of performing the test, since reduced haemoglobin is formed which obscures the haemochromogen bands. In addition specimens of plasma were examined in Harrison's (1938) apparatus to a depth of 6 to 10 cm. for spectroscopic evidence of methaemalbumin.

Preparation of Haemoglobin Solutions.—The haemoglobin used for the injections was prepared from citrated normal human blood; the plasma was pipetted off after centrifugalization and the corpuscles washed in physiological saline. The sedimented corpuscles were then haemolysed by adding four times the volume of sterile doubly distilled water and stromata were removed by centrifugalization. Sterile NaCl was added to make the haemoglobin solution isotonic with blood; it was subsequently left in the ice-room overnight and centrifuged next morning, when any remaining stromata were separated. The haemoglobin was finally estimated by Haldane's method, 100% corresponding to 13.8 grammes haemoglobin per 100 c.cm.

Case Histories

The patients receiving these haemoglobin injections were all convalescent. Preliminary alkalization of the urine was established before the injections, and in each case the urine was kept alkaline to litmus for the next twenty-four hours by the hourly or two-hourly administration of a potassium citrate and sodium bicarbonate mixture.

Case 1.—A man aged 23 had been admitted to hospital with an injury to his thumb and ankylostomiasis, which was successfully treated. He received 14 grammes of haemoglobin, or 280 mg. per kg. of body weight, intravenously. No untoward reaction followed. The injection took fifteen minutes, and at completion blood was withdrawn and the plasma found to contain 208 mg. haemoglobin per 100 c.cm. of plasma. Thereafter the haemoglobinaemia progressively decreased, as shown in the graph (Case 1). Schumm's test was positive in

a quarter hours; no haemoglobin was visible spectroscopically in any subsequent specimens—even in a depth of 10 cm. The blood urea varied between 32 and 36 mg. per 100 c.cm. throughout the experiment. Bilirubin estimations showed a rise to 1 unit (indirect) about three and a half hours after the haemoglobin was injected and 0.5 unit at ten hours. It subsequently fell to 0.1 unit, as shown in the graph.

Case 2.—A man aged 49 was admitted to hospital with cirrhosis of the liver, intermittent claudication, and a streptococcal infection of the urinary tract. The last-named was effectively treated with prosectasine. The patient received 21 grammes of haemoglobin, or 280 mg. per kg. of body weight, intravenously. The injection took fifteen minutes; blood was then withdrawn and the plasma was found to contain 230 mg. of haemoglobin per 100 c.cm. The subsequent fall in haemoglobin concentration is shown in the graph (Case 2). The first specimen of urine was passed thirty-five minutes after the injection and was found to be alkaline to litmus and to contain much oxyhaemoglobin and a few granular casts, but no methaemoglobin. Haemoglobinuria persisted for 8 hours 50 minutes. During this period the urine was invariably alkaline to litmus, and on the last four occasions contained a trace of methaemoglobin, though this was only visible spectroscopically when examined through a depth of 6 cm. Schumm's test was first found to be positive 5 hours 50 minutes after the injection began, and continued to be positive for twenty-two and a half hours, the α band at 558 $m\mu$ being demonstrable spectroscopically through a thickness of 0.1 to 2 cm. of plasma. When next examined at twenty-eight hours the reaction was negative. Methaemalbumin was never demonstrable spectroscopically in plasma even in a depth of 6 cm. The bilirubin content of the plasma was 0.5 unit (indirect) before the injection; it rose to a maximum of 1.25 units five and three-quarter hours later. Nausea and mild fever (100° F.) developed some hours after the injection, but the temperature was normal in twenty-four hours, and nausea disappeared when the bicarbonate-citrate mixture was stopped.

Case 3.—A man aged 35 years was admitted to hospital with ascariasis and syphilis, for which he received effective treatment. An injection of 17.4 grammes of haemoglobin, or 350 mg. per kg. of body weight, was given intravenously. No adverse reaction followed the injection, which took thirty minutes. Blood collected at this time showed 230 mg. of haemoglobin per 100 c.cm. plasma. Thereafter the haemoglobinaemia decreased as shown in the graph (Case 3). Some five hours after starting the first injection a second intravenous injection of 8 grammes of haemoglobin, or 160 mg. per kg., was given. Immediately following this injection the haemoglobin in the plasma rose from 106 mg. to 198 mg. per 100 c.cm. The first specimen of urine was obtained in two hours; it was alkaline to litmus and contained oxyhaemoglobin and methaemoglobin. Haemoglobinuria persisted for at least sixteen hours. During this period the urine remained alkaline; oxyhaemoglobin was always present and methaemoglobin invariably absent. The plasma gave a doubtful Schumm reaction seven hours after injection and was strongly positive at ten hours, the α band of haemochromogen being evident in a thickness of 0.2 cm. This remained positive for thirty-four hours. The rise in bilirubin was so slight as to have no significance. The blood urea varied between 32 and 36 mg. per 100 c.cm.

Comment

The number of cases investigated along these lines was curtailed by the outbreak of war, otherwise the series would have been extended and larger quantities of haemoglobin injected in order to determine what amount was necessary to produce a concentration of methaemalbumin sufficient to be demonstrated spectroscopically.

Haemoglobinaemia.—The amount of haemoglobin injected was 280 mg. per kg. of body weight in the first two patients, while the third received two injections—350 mg. and 180 mg. per kg. The maximal concentration of haemoglobin at the end of the injections varied from 208 to 230 mg. per 100 c.cm. The fall was subsequently rapid, but persisted in sufficient concentration to give rise to haemoglobinuria lasting 7 hours in Case 1, 8 hours

Graph showing results of intravenous injections in three cases.

plasma collected 5 hours 10 minutes after the injection, and it remained strongly positive up to twenty-eight hours, the α band at 558 $m\mu$ being clearly defined in a depth of from 0.1 to 1 cm. No further observations were made. Methaemalbumin was never demonstrated spectroscopically. The haemoglobinuria was found within thirty-five minutes and disappeared after seven hours. The first specimen of urine was slightly alkaline to litmus and contained oxyhaemoglobin, a trace of methaemoglobin, and a few granular casts. The second specimen also contained both oxyhaemoglobin and methaemoglobin; but the remainder, which were all definitely alkaline to litmus, showed only oxyhaemoglobin up to seven and

50 minutes in Case 2, and 16 hours in Case 3, the recipient of the two injections. The quantity of the haemoglobin present in the plasma at the termination of the haemoglobinuria was 69 mg. in Case 1, 51 mg. in Case 2, and 37 mg. in Case 3. The renal threshold for haemoglobin lay somewhere between 37 and 102 mg. per 100 c.cm. for the series, but more extended observations would be necessary to establish this value with reasonable accuracy.

Bilirubin.—The bilirubin determinations were not considered satisfactory for the technical reasons already given, but quite definite and sustained increases in bilirubin were observed in Cases 1 and 2, which confirmed the observations of Duesberg in this regard. Furthermore, the elevation of bilirubin in Case 2, with cirrhosis of the liver, lasted twenty-two hours and was more prolonged than in Case 1, which lasted ten hours; this finding was not unexpected, since the concentration of indirectly reacting bilirubin (haemobilirubin) in the blood is dependent not only on the rate and amount of blood destruction, but on the functional capacity of the liver to excrete the circulating haemobilirubin as bile pigment. The rise in bilirubin in Case 3 was too small to be of any significance.

Schumm's Test.—In contradistinction to Duesberg's negative findings it will be observed that the plasma of all three cases gave a positive reaction within four to ten hours, and that this persisted for twenty-seven to thirty-four hours from starting the injection, the α band of haemochromogen being demonstrable at 558 $m\mu$ in a thickness of from 0.1 to 2 cm. of plasma. Duesberg (1934) used a maximum of 6 grammes of haemoglobin for his patients weighing 75 kg., and the disparity in results must be attributed to the larger quantities of haemoglobin injected, which in the present series of cases were 14, 21, and 25.4 grammes respectively.

Methaemalbumin.—In all three cases the plasma was examined with the reversion spectroscopie for the presence of methaemalbumin in a maximum depth varying from 6 cm. to 10 cm. An α band in the red portion of the spectrum was never observed. No opportunity has been forthcoming for injecting larger quantities of haemoglobin, but in two patients (Cases 4 and 5) receiving incompatible blood transfusions containing approximately 90 and 45 grammes of haemoglobin respectively, methaemalbumin was demonstrable spectroscopically in both instances.

The demonstration of methaemalbumin, whether spectroscopically or by Schumm's test, is dependent on its concentration in the plasma, but the latter method is more sensitive than the former. The concentration is determined by the amount of circulating extracorporeal haemoglobin and the efficiency of the various mechanisms for its elimination, which will be referred to later. Once methaemalbumin has formed its elimination appears to be via the liver, dysfunction of which results in an increased concentration in the blood.

II. INCOMPATIBLE TRANSFUSION

Two cases of incompatible transfusion were investigated. In one the recipient (Group O (IV Moss)) was transfused with a Group B (III) blood; owing to depreciation in titre of the Group A (II) typing serum the donor was classified erroneously as Group O instead of B. In the other case the typing error was not determined with certainty.

Case 4.—A man aged 68 was gravely ill with what had been regarded as a post-sprue megalocytic anaemia. R.B.C., 960,000 per c.mm.; haemoglobin, 12% (Haldane); colour index, 0.7. Average diameter of corpuscle, 8.6 μ . Megalocytosis marked. Bilirubin, 1.5 units (indirect); blood urea, 33 mg. per 100 c.cm. Shortly after being transfused with 37½ ounces of citrated blood the temperature rose to 102.6° F., and subsequently port-

wine-coloured urine was passed containing oxyhaemoglobin and methaemoglobin. Some fourteen and a half hours after transfusion the patient was jaundiced; the bilirubin reached 12 units (indirect) and the blood urea 68 mg. per 100 c.cm.; thirty-nine hours later the bilirubin equalled 10.5 units and the blood urea 132 mg. Examination of the plasma on the reversion spectroscopie showed a three-banded spectrum with an α band in the red at 623 $m\mu$, a β band at 578 $m\mu$, and a γ band at 540 $m\mu$. This resembled methaemoglobin, but was really a composite spectrum, the α band being due to methaemalbumin and the β and γ bands corresponding to the α and β bands of oxyhaemoglobin. The patient died with anuria within forty-eight hours of the transfusion. The post-mortem diagnosis was pernicious anaemia complicated by intravascular haemolysis associated with an incompatible blood transfusion. The heart was large (16 oz.), fatty, and flabby; the liver (68 oz.) was ochre-yellow and fatty; and the gall-bladder was greatly distended with thick black bile the consistency of treacle. Early consolidation of the base of the right lung was present. Histopathological investigation showed typical blockage of the renal tubules with eosinophilic debris, some degenerative changes in the lining tubular epithelium, and much haemosiderin in the parenchyme cells of the spleen, liver, and kidneys.

Case 5.—A woman aged 55 was admitted with anaemia following haemorrhage from a fibroid. On the night of June 22, 1937, when a blood transfusion was being given previous to operation, she became shocked, and developed tightness in the chest, a rash on the arms, and swelling of the hands. Three extremely loose bowel actions followed. Next morning the patient, who was passing dark red urine, was jaundiced and very ill. Laboratory investigations were made on June 23. The plasma obtained in the morning was a dark golden-brown colour. There was the usual three-banded spectrum, with an α band at 623 $m\mu$, a β band at 578 $m\mu$, and a γ band at 540 $m\mu$. Unlike the α band of methaemoglobin it was not dispersed with Stokes's reagent or 10% ammonium sulphide; it was completely dispersed with strong ammonium sulphide, but formed a haemochromogen with the prominent α band at 558 $m\mu$. Bilirubin amounted to 2.5 units (indirect), and the blood urea to 100 mg. per 100 c.cm. The urine at 5.30 p.m. was very dark red in colour, was acid in reaction, and contained 84 mg. of haemoglobin and 724 mg. of methaemoglobin per 100 c.cm. The deposit showed hyaline, granular, and epithelial casts. Plasma collected at the same time revealed methaemalbumin. Blood urea amounted to 115 mg. per 100 c.cm. On June 24 urine collected at 7 p.m. was alkaline and still contained oxyhaemoglobin but no methaemoglobin. Next day the plasma contained no oxyhaemoglobin or methaemalbumin, and the blood urea was 168 mg. per 100 c.cm. After a severe illness the patient recovered.

III. INTRAVENOUS INJECTIONS OF HAEMATIN IN MAN AND MONKEYS

In a recent series of observations on man and monkeys receiving intravenous injections of alkaline haematin (ferric)* the finding of Duesberg (1934) that there was no increase in circulating bilirubin was confirmed by me. Duesberg's findings regarding experimentally induced haematinæmia in man were, however, not verified, inasmuch as the pigment present in the circulating blood proved to be methaemalbumin and not haematin. The experimental evidence supporting this view is being reported in another communication, but it might be pointed out here that the spectrum was typical of methaemalbumin, the α band being situated at 623 $m\mu$ and not at 616 $m\mu$, which was the position of the α band in the alkaline haematin injected. The α band in the red was not altered by Stokes's reagent or 10% ammonium sulphide, but it was dispersed with concentrated ammonium sulphide, a haemochromogen being formed with a well-defined α band seen at 558 $m\mu$ (Schumm's test).

* The alkaline haematin (ferric) was prepared by adding just enough sodium hydroxide or sodium carbonate to pure haematin in saline to dissolve it completely. The dosage in man was 4 mg. per kg. and in monkeys 12.5 to 20 mg. per kg. of body weight.

The pigment was also found to be concentrated in the albumin fraction of the serum, and on reduction with sodium hydrosulphite yielded the typical double-banded spectrum of haemalbumin (ferrous), and not the broad single-banded spectrum of reduced haematin. On treatment with sodium hydrosulphite and CO, carboxyhaemalbumin was formed, having a spectrum which closely resembled but was not identical with carboxyhaemoglobin. On treatment with sodium hydrosulphite and 10% sodium hydroxide a haemochromogen with an α band at 558 m μ was produced.

Identical results were obtained with monkeys of the species *Macacus rhesus*, *Macacus sinicus*, and *Cercopithecus aethiops* injected with alkaline haematin (ferric). When injections of reduced alkaline haematin (ferrous) were given intravenously, methaemalbumin also appeared; evidently the reduced haematin (ferrous) was oxidized in the blood stream to haematin (ferric), which united with serum albumin to form methaemalbumin.

IV. DISCUSSION

The conclusion reached from these investigations as well as from an extensive clinical study of the haemoglobinurias and haemolytic anaemias is that extracorporeal circulating haemoglobin is treated as a foreign substance in the body, which disintegrates and eliminates it in as short a time as possible. Three mechanisms, depending on the concentration of extracorporeal haemoglobin attained in the circulation, may be called into action for this purpose. They are: (1) absorption by the reticulo-endothelial system; (2) intravascular katabolism of haemoglobin; (3) renal excretion.

Absorption by the R.-E. System.—Effete or damaged corpuscles are phagocytosed and circulating extracorporeal haemoglobin is absorbed by the reticulo-endothelium, the end-products of intracellular katabolism of the haemoglobin being bilirubin and haemosiderin. Lemberg (1937) has suggested that haemoglobin is first transformed in R.-E. cells into an unstable protein-haemochromogen which is rapidly oxidized to verdo-haemochromogen. The latter compound then loses its iron together with protein, which may remain combined to give rise to iron-containing "haemosiderin" proteins. Thus biliverdin is formed and undergoes reduction to bilirubin partly in the R.-E. cells and the remainder in liver cells. With slight degrees of intravascular haemolysis, or where only small amounts of haemoglobin are injected into the circulation (5 grammes), this is probably the only mechanism involved. In such circumstances biochemical investigation shows some degree of hyperbilirubinaemia, a negative Schumm test, and an absence of methaemalbuminaemia.

Intravascular Katabolism of Haemoglobin.—Where intravascular haemolysis is more marked, biochemical investigation reveals not only hyperbilirubinaemia but also a positive Schumm test and methaemalbuminaemia. The concentration of these pigments is increased if there is an associated degeneration of the polygonal cells of the liver. In addition to the intracellular disintegration of haemoglobin already referred to there is now an extracellular katabolism of haemoglobin in the circulation; haematin (ferric) is formed, combining with serum albumin to form methaemalbumin. When human plasma-haemoglobin mixtures are incubated at 37° to 40° C. neutral and alkaline methaemoglobin are demonstrable before methaemalbumin appears; this suggests that the extracorporeal haemoglobin may be first oxidized to methaemoglobin, which is subsequently split into globin and haematin (ferric), the haematin uniting with serum albumin to form methaemalbumin. On the other hand, in a severe intravascular haemolysis, such as

occurs in blackwater fever, no spectroscopic evidence is available that methaemoglobin in demonstrable quantity does precede the formation of methaemalbumin. In monkeys receiving intravenous injections of reduced haematin (ferrous) methaemalbumin appears in the blood. It may therefore be that, *in vivo*, circulating extracorporeal haemoglobin is first split into globin and reduced haematin (ferrous), and the latter pigment is subsequently oxidized to haematin (ferric), which immediately combines with serum albumin to form methaemalbumin. The balance of evidence so far available favours this view. Rimington (1939) has found in both man and animals that faecal porphyrin is increased after haematin injections, and has suggested that the prosthetic group of methaemalbumin is eliminated via the liver as Series III porphyrin in the faeces.

Renal Excretion.—The third mechanism to be brought into play is renal excretion of oxyhaemoglobin, and, as is well known, haemoglobinuria develops only when the concentration of extracorporeal haemoglobin is sufficient to exceed the renal threshold. Baker and Dodds (1925) found on injecting haemoglobin into rabbits that, provided the urine was alkaline in reaction, the oxyhaemoglobin remained in solution unchanged and no blockage of the tubules resulted. Clinical observation supports this view. When the urine is acid, methaemoglobin and, later, acid haematin are formed. Haematin is deposited in the tubules either as acid haematin or as haematin combined with some nitrogenous base or protein, and if sufficient nephrons are blocked anuria results. Anuria with uraemia is the common cause of death in any severe intravascular haemolysis in man. For this reason routine preliminary alkalization of the urine is a sound procedure in all blood transfusions, while the immediate alkalization by massive intravenous injections of citrates, as recently advocated by Bushby, Hart, Kekwick, and Whitby (1940), is indicated in any condition immediately haemoglobinuria appears. The lack of untoward symptoms or severe reaction following the injection of human haemoglobin in a dosage of 280 to 350 mg. per kg. of body weight is of interest, and is undoubtedly related to the alkalization of the urine which was carried out before the injection in each case.

Summary and Conclusions

Following the intravenous injection of 14 to 25.4 grammes of human haemoglobin in man a positive Schumm reaction was invariably demonstrated in the plasma within four to ten hours, and persisted for twenty-four hours or longer.

A transient increase in bilirubin was also noted in the plasma in two of the three cases receiving haemoglobin injections.

Though methaemalbumin was not found spectroscopically following the injection of haemoglobin in these amounts, it was demonstrated in the plasma of two patients who received by mistake incompatible blood containing approximately 45 and 90 grammes of haemoglobin respectively.

In incompatible transfusion and the other intravascular haemolyses in man, methaemalbumin and not methaemoglobin is found in the plasma; a mixture of the two pigments has never been recorded *in vivo*, though they do occur together *in vitro* when a human plasma-haemoglobin mixture is incubated at 37° C.

Extracorporeal circulating haemoglobin may be (a) excreted by the kidney, (b) absorbed by the R.-E. cells with the production of bilirubin and haemosiderin, and (c) katabolized in the circulation with the production of methaemalbumin.

It is suggested that haemoglobin disintegrating in the circulation is first split into globin and reduced haematin (ferrous); the latter pigment is immediately oxidized to haematin (ferric), which in man combines with serum albumin to form methaemalbumin.

I wish to express my indebtedness to Dr. C. M. Wenyon, F.R.S., for laboratory facilities for continuing this work at the

Wellcome Research Institution after the closure of the Hospital for Tropical Diseases, London, and to the Colonial Office for a research grant which has enabled me to retain the valuable services of Mr. R. J. Bromfield in completing the investigation.

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A RAPID METHOD OF DIFFERENTIATING CHILDREN WITH LARGE OR SMALL RESERVES OF VITAMIN C*

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Previous tests of adequacy of vitamin C storage based on urine analysis have in general been unsuitable for wide use on subjects other than hospital patients owing to the desirability of testing a twenty-four-hour specimen. Abbasy and Harris (1937a) suggested a modification of their original method in which three-hour specimens were tested on two successive days. On the third day, and the fourth if necessary, they gave a test dose of ascorbic acid, and the urine passed four to seven hours after was analysed. The present experiments were carried out to discover if the measurement of the concentration of vitamin C in a single specimen of urine passed four hours after a test dose of ascorbic acid would reveal reliable information regarding the vitamin C reserves of the subject tested.

Experiment on Concentration of Vitamin C in Urine of Normal Adult

The first of the two experiments was designed to show the variations in concentration of vitamin C in the urine from specimen to specimen and from day to day in a normal healthy adult male before and after a test dose of ascorbic acid. This subject (J. P.) had a diet which included fresh fruit at least once a day and which contained on the average approximately 70 mg. of ascorbic acid daily. This figure was derived from an analysis of a detailed record of the diet for one week. Each specimen of urine passed during a period of seventeen days was measured and its concentration of vitamin C determined. On the ninth day a test dose of ascorbic acid—700 mg.—was taken by mouth. The data derived from this experiment are summarized in Tables I and II and in Chart 1. It will be seen that the average concentration of vitamin C in the specimens before the test dose was 1.72 mg. per 100 ml. In 76 per cent. of these specimens the concentration lay between 0.76 and 2 mg. per 100 ml. When the concentration rose to levels of 3 to 6 mg. per 100 ml. extra quantities of fruit had been consumed. That the concentration of vitamin C is not directly dependent on the volume of the specimens is shown in Table II. The effect of a large test dose of ascorbic acid (70 mg. per stone of body weight) on the concentration of vitamin C in subsequent specimens of urine is shown in Chart 1.

* This research was carried out in connexion with the Carnegie United Kingdom Trust dietary and nutritional survey.

TABLE I.—*The Excretion of Vitamin C by an Adult Male Weighing 10 st. on a Daily Intake of Approximately 70 mg. and the Response to a Test Dose of 700 mg. Ascorbic Acid*

Duration of observation period	17 days
Number of specimens of urine passed	114
.. before test dose (8 days)	55
Average daily urinary excretion	1,495 ml.
Range in volume of specimens	40 to 520 ml.
Average concentration of vitamin C in specimens before test dose of ascorbic acid	1.72 mg. per 100 ml.
Range in concentration of vitamin C in specimens before test dose	0.76 to 6.5 mg. per 100 ml.
Total urinary excretion, showing the diuretic effect of ascorbic acid:	
On day of test dose	2,140 ml.
On day following test dose	1,980 ml.
Average total daily excretion of vitamin C before test dose (8 days)	22.8 mg.
Range of total daily excretion of vitamin C before test dose	13.8 to 29.1 mg.
Maximum concentration reached 6 hours after test dose	27.7 mg. per 100 ml.
Total amount of vitamin C excreted in 12 hours following test dose	245.1 mg.
Percentage of test dose excreted in 12 hours following administration	35%

TABLE II.—*Showing Lack of Relation between Volume of Specimen and Concentration of Vitamin C*

Time	Volume of Specimen (ml.)	Concentration of Vitamin C (mg. per 100 ml.)
1st day:		
8.30 a.m.	485	1.23
9.45 a.m.	55	1.21
12.30 p.m.	155	1.49
1.45 p.m.	75	1.63
4.30 p.m.	175	1.84
6.0 p.m.	110	1.41
8.0 p.m.	90	2.83
11.15 p.m.	580	1.43
2nd day:		
8.30 a.m.	400	1.94
12.30 p.m.	110	2.11
2.0 p.m.	90	1.85
4.50 p.m.	215	1.85
6.0 p.m.	175	0.89
7.0 p.m.	145	0.77
9.30 p.m.	460	1.91
10.0 p.m.	95	4.67

CHART 1.—Showing the variations in concentration of vitamin C in successive specimens of urine in a male adult before and after a test dose of ascorbic acid.

Following the marked increase in concentration of vitamin C in the urine on the day of the test dose the concentration fell rapidly, and on the third day after the test dose the concentration in five out of six specimens was less than 2.3 mg. per 100 ml. By the eighth day all the concentrations were less than 2 mg. per 100 ml., and the total daily excretion was 19.9 mg. During the week following the test dose increases up to 7 mg. per 100 ml. occurred on several occasions after the consumption of small amounts of fruit, showing a high degree of saturation in the tissues.

Method of Analysis Used

The method of analysis employed in determining the concentration of vitamin C in the urine was the same as that described by Harris and Ray (1935). Each specimen