CLXXXIV. THE ROLE OF VITAMIN C IN ANIMALS RESISTANT TO SCURVY: EFFECTS OF INSULIN AND ADRENALINE.

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THE recent knowledge of the relation between vitamin C and the carbohydrate group of substances made it of interest to investigate any effect, on that vitamin, of substances such as insulin and adrenaline which have an influence on the storage, mobilization or utilization of carbohydrates in the animal body. Such an investigation necessitates the comparison of the amounts of the vitamin in the chief organs which contain it as well as the amounts excreted by animals before and after the administration of the substances.

It is well known that animals are divided into two classes, those affected by the deficiency of the vitamin in the diet and those which can withstand the deficiency. It is questionable which of the two classes will give more fruitful results under the conditions of the investigation. Among animals of the second class are rats, and it has been suggested by Parsons [1920], Parsons & Hutton [1924], Lepkovsky & Nelson [1924], and Parsons & Reynolds [1924] that the capacity of the rat for living without vitamin C may be due to the fact that these animals can synthesize it. Since it is aimed to include in this work a study of the possible influence of the substances above mentioned on the synthesis of vitamin C from precursors in the body, it was thought preferable to use animals which can synthesize the vitamin.

It is obvious that, unless the amounts of vitamin excreted and stored by such animals are controlled before the administration of the drugs, no reliable results can be obtained. At the same time an exact knowledge of the role of vitamin C in animals which are non-susceptible to scurvy when kept on a scorbutic diet is at present lacking. It was, therefore, planned to start the present investigation by the study of the storage and excretion of the vitamin in rats kept on an ordinary mixed diet and on a scorbutic diet for different periods, hoping at the same time to determine the best conditions for attaining instructive results about the influence of adrenaline and insulin on that vitamin.

EXPERIMENTAL PROCEDURE.

Certain organs and glands of animals, in general, contain vitamin C in higher concentration than most other tissues. The suprarenals and the liver have repeatedly been shown to be rich in the vitamin; further, the work of McCarrison [1919] shows that the suprarenals have a special physiological connexion with the vitamin. Although the concentration of the vitamin in the liver is smaller than in the suprarenals, yet, by virtue of the large bulk of the

former its total content is far greater than that of the latter. The procedure adopted in this investigation is confined to the determination of the vitamin C content of the suprarenals, liver and the 24 hours' urine under different conditions. The method applied for the estimation of the vitamin in the organs and in the urine is the modification made by Emmerie & Van Eekelen [1934] of the methods of Birch *et al.* [1933] and Tillmans *et al.* [1932], which includes the removal of the substances interfering with the titration with 2:6-dichlorophenol-indophenol by mercuric acetate followed by reducing the portion present in the oxidized form by hydrogen sulphide and titrating the total vitamin, in the reduced form, by means of a solution of the indicator standardized against a solution of pure ascorbic acid. The determination of the total vitamin, both oxidized and reduced, gives more accurate information, as it is possible that a portion of the reduced form undergoes oxidation during extraction from the organs or in the urine on standing, and, as has been shown by Tillmans *et al.* [1932] and by Hirst & Zilva [1933], the reversibly oxidized form of ascorbic acid has a similar activity to the reduced form.

Working with rats, however, presents certain difficulties. The suprarenals of a rat are too small to give, after the different manipulations involved in the above method, a sufficient volume of an extract of a reasonable concentration for the purpose of determination, but this difficulty was surmounted by using the four suprarenals obtained from 2 rats for each experiment and by modifying, by means of preliminary experiments, the proportions of the reagents to be used and the procedure to be adopted. All the rats used for the experiments were males taken from a stock which had been bred in the laboratory for many generations; the age difference did not exceed 7 days and the difference in weight between the animals did not exceed 10 g. They were kept and treated in exactly the same manner before being used for the experiments. After killing the animals by cutting the spinal cord, the four suprarenals were rapidly removed and prepared for the estimation of the vitamin.

In the case of the liver, the two livers obtained from the 2 rats were used for the extraction and determination of the vitamin.

The greater difficulty was met with in the case of the determination of the vitamin in the 24 hours' urine, as some factors interfere with the results. The first factor is the inexactness of the volume of 24 hours' urine due to varying amounts remaining in the bladder at the close of the period, but such an error is minimized by conducting a number of experiments and prolonging the time of observation under the same conditions whenever possible. The second factor is that the urine voided by rats may sometimes be alkaline or may turn so later, thus causing instability of the vitamin on standing. This difficulty was overcome by placing a measured volume of 20% acetic acid in the receivers and allowing for that volume in the calculations. The third factor is the change which the vitamin undergoes on standing. This factor is also minimized by acetic acid; but it has been found that ascorbic acid, even in acid medium, undergoes two sorts of change to a slight extent; one is reversible oxidation and the other is apparently destruction. Since the method of reduction before estimation was adopted, the oxidation did not matter. As regards the destruction, this was determined under the same conditions as were adopted for the collection of urine from the animals and was found not to exceed 14% at various air temperatures. It may be remarked that in the case of collection from rats the total destruction will be less than this, since the urine is voided at different intervals and therefore the time which elapses before the estimation is made is actually less than 24 hours for a considerable bulk of the urine. It has also been found that, even if the destruction amounts

to 14%, it does not seriously affect the interpretation of the results. Nevertheless, only limits of variations in a number of experiments, performed in the same manner and extended over long periods whenever possible, have been taken into consideration in the interpretation of the results.

Observations on vitamin C of the suprarenals and liver of rats.

A large number of rats weighing 220–230 g. and chosen as previously indicated were placed on the scurvy-producing diet recommended by Hassan & Basili [1932]. All rats kept on that diet gained in weight at the rate of 4–5 g. per rat per week. The vitamin contents of four suprarenals and two livers taken from 2 rats, which were killed at the same time and were chosen of exactly the same weight at the time of killing, were determined after different periods on the scorbutic diet. Similar rats to those used for each experiment but kept on ordinary mixed diet were also used for the determination of the vitamin contents of the same organs. The diet used for the latter animals was composed of equal amounts of bread, fresh clover and milk. The bread and clover were minced together and the milk was then added. This diet will be referred to in the text as ordinary diet. Table I gives the data obtained for the rats kept on the scorbutic diet for different periods.

It will be noticed that the total content of vitamin of the livers and of the suprarenals does not undergo any change up to 3 months, but, whilst the concentration of the vitamin, calculated in mg. per g., is almost constant in the livers, it shows considerable variations in the suprarenals. The gradual increase in the body weight of the rats, which amounts to 4–5 g. per rat per week during the 90 days on the scorbutic diet, has no effect on the vitamin content of the organs.

Table I.

Period on scorbutic diet days	Vitamin per g. suprarenals mg.	Total vitamin in 4 suprarenals mg.	Vitamin per g. liver mg.	Total vitamin in two livers mg.
3	4.60	0.23	0.24	3.54
6	3.92	0.22	0.24	3.66
9	2.27	0.24	0.22	3.39
13	2.18	0.20	0.23	3.52
18	2.27	0.23	0.23	3.72
23	2.36	0.22	0.22	3.52
28	2.65	0.21	0.24	3.72
35	2.87	0.20	0.23	3.70
45	3.61	0.22	0.20	3.37
55	3.59	0.20	0.19	3.29
65	4.60	0.23	0.20	3.56
75	4.58	0.21	0.19	3.64
90	4.22	0.23	0.21	3.66
Limits	2.18–4.60	0.20–0.24	0.19–0.24	3.29–3.72

The variations noticed in the concentration in the case of the suprarenals are ascribed to changes in the weights of these organs at different periods. It was observed that the suprarenals show a considerable increase in size and in weight between the 6th and 9th days up to the 28th day on the scorbutic diet. This is clearly indicated by comparing the concentrations of the vitamin in the suprarenals during the different periods (column 2, Table I). It may be remarked that this increase in the weight of the suprarenals is not in proportion to the body weight; this is evident from the comparison of the weights of the suprarenals during the different periods on a scorbutic diet with each other and with

the weight of those of rats of the same weight but kept on the ordinary diet (columns 1 & 4, Table II). All weights are expressed in mg. per g. of body weight for the purpose of eliminating the factor which might be introduced by the latter in the comparison during the different periods, since the rats gradually increase in weight.

Table II.

Rats on scorbutic diet			Rats on ordinary diet		
Suprarenals per g. body weight mg.	Vitamin in 4 suprarenals mg.	Vitamin in two livers mg.	Suprarenals per g. body weight mg.	Vitamin in 4 suprarenals mg.	Vitamin in two livers mg.
0.116-0.124 (1-6 days)	—	—	—	—	—
0.132-0.223 (between 6 and 9 up to 28 days)	0.20-0.24	3.29-3.72	0.113-0.124	0.19-0.23	3.66-3.74
0.118-0.122 (28-90 days)	—	—	—	—	—

In view of the observation of McCarrison [1919] that the suprarenals of guinea-pigs in scurvy show an increase in size and in weight, the occurrence of this increase in the case of rats on a scorbutic diet was repeatedly confirmed. It extends only over a certain period and is greater about the beginning than at the close of the period. The nature of this increase is still under investigation, but it can be said at present that it does not appear to be a hypertrophy due to increase in the synthesis of the vitamin by these organs.

Table II gives a comparison of the total content of the vitamin in the suprarenals and livers of rats kept on a scorbutic diet for different periods with the vitamin contents of similar rats kept on the ordinary diet. The experiments show that the contents in the latter case are practically constant, regardless of the gradual increment of the weight of the animals, and that these contents do not alter in magnitude in rats kept on a scorbutic diet up to 90 days.

Vitamin C in the urine.

Rats of the same age and weight were placed on the ordinary diet. Every pair was placed in a Hopkins metabolism cage and the amount of food consumed by the pair in 24 hours was determined for some days. Every pair was then given an amount of the diet which would be completely consumed in 24 hours without causing inanition. After 1 week on the fixed amount of the diet, the urine was collected daily for the determination of the vitamin excretion in 24 hours. Five experiments were performed in this manner and the determinations were extended in each case for 15 days. After the close of this period, the diet was replaced by the scorbutic diet and the determinations were continued for 90 days. For the sake of brevity the results of the five experiments are represented in Fig. 1 where the limits of variation in the excretion of the vitamin per rat in 24 hours on the two diets for different periods are given.

The results show that in every case the excretion is variable. Taking the limits of variation into consideration, however, it is noticeable that, after the replacement of the ordinary diet by the scorbutic diet, there is a rapid fall in the vitamin C excretion which, in 48 hours, becomes less than half its previous value; thus, while the limits of variation in the excretion on the ordinary diet are from 0.85 to 1.22 mg. per rat per 24 hours, the limits from the 2nd to the 9th day on the scorbutic diet are from 0.34 to 0.55 mg. After the 9th day on the scorbutic

diet there is a somewhat gradual increase in the excretion; thus, during the next 6 days the limits are from 0.55 to 0.825 mg. per rat per 24 hours. From the 16th to the 90th day the limits are from 0.60 to 1.08 mg., approaching on some

Fig. 1.

occasions the higher limit of excretion reached in the case of the ordinary diet; the level of excretion during this period is generally lower than in the case of the ordinary diet.

Effect of insulin on storage and excretion of vitamin C.

The above experiments show that the vitamin C content of the suprarenals and livers of rats is constant whether these animals are kept on ordinary mixed diet or on a scorbutic diet for different periods, whilst the excretion in the urine shows variations, the limits of which under the different circumstances are known in each case. It was, therefore, thought advisable to administer the insulin to rats which had been kept on a scorbutic diet for more than 16 days so that the variations in the excretion would have attained certain limits. All the rats used were kept on the scorbutic diet for $2\frac{1}{2}$ months or more. The question also arose whether to give or to withhold the diet after the insulin injections. It is well known that insulin causes the animals to ingest more food if diet is allowed and hence a new factor, which could not previously be accounted for, would be introduced. It was, therefore, preferred to withhold the food during the 24 hours following the insulin injection. It then became necessary to determine the influence of withholding the diet for 24 hours on the vitamin content of the suprarenals and of the liver as well as on the amount excreted in the urine of rats previously kept on the scorbutic diet for the period indicated. The influence of insulin was also tried in some cases when diet was given.

A comparison of the vitamin contents of four suprarenals and two livers of 2 rats when starved for 24 hours with the usual content of these organs of two animals which were allowed to feed till the time of killing is given in Table III.

Table III.

Exp.	Starved for 24 hours		Diet allowed	
	Total vitamin in 4 suprarenals	Total vitamin in two livers	Total vitamin in 4 suprarenals	Total vitamin in two livers
	mg.	mg.	mg.	mg.
1	0.22	3.56	0.20	3.29
2	0.21	3.49	0.24	3.72

While starvation for 24 hours does not alter the vitamin C content of the suprarenals or of the livers it has a rather unexpected effect on the amount excreted in the 24 hours' urine. Thus while the amount excreted in 24 hours usually varies from 0.60 to 1.08 mg. per rat and never exceeds the higher limit, during starvation for 24 hours the limits of variations are from 1.10 to 1.35 mg. per rat, i.e. the excretion always rises (frequently to about 23 %) above the usual higher limit.

The results of the four experiments, graphically represented in Fig. 2, give strong support to this observation. 8 rats, previously kept on the scorbutic diet for about 2½ months, were placed in metabolism cages—one pair in each cage—and the scorbutic diet was continued. The vitamin excretion was determined and was found to be within the usual limits. The pairs were alternately deprived of the diet for 24 hours. The arrows indicate when the diet was stopped for 24 hours and each graph represents the excretion of one pair.

Fig. 2. ↑ Diet stopped for 24 hours.

It was next found that 4 units of insulin when injected into rats of 270–275 g. caused convulsions after about 10 or 11 hours and then death. Three units also caused similar effects, but a little later. The susceptibility to insulin was found to be the same whether the animals were previously kept on a scorbutic diet or on an ordinary diet. It was found that 2 units of insulin could be safely administered to rats of the above-mentioned weight when no diet was given.

Each pair of rats of the same age and weight, which had been kept on the scorbutic diet for about 2½ months, was placed in a metabolism cage. They were allowed to feed and the amount of the vitamin in the 24 hours' urine was determined daily in order to make sure that the excretion of these particular animals

was within the usual limits. No abnormality was observed in any of the experiments. On the morning of one day 2 units of insulin were injected into each rat and no diet was given. After 23 hours, i.e. 1 hour before the rats were killed, 3 more units were injected into each rat. At the close of the 24 hours the rats were killed and the vitamin C contents of the suprarenals, liver and 24 hours' urine were determined. Since the contents of the organs were proved not to be altered by fasting for 24 hours, their content after the administration of insulin is compared with the usual content. The excretion in urine, however, is compared with the excretion of similar rats when fasted for 24 hours (Table IV).

Table IV.

Exp.	Vitamin in 4 suprarenals mg.		Vitamin in two livers mg.		Vitamin excreted per rat in 24 hours mg.	
	Insulin	No insulin	Insulin	No insulin	Fasting and insulin	Fasting and no insulin
1	0.20		5.46		1.20	
2	0.21		5.01		0.98	
3	0.20	0.20-0.24	5.32	3.29-3.72	1.15	1.10-1.35
4	0.22		5.43		1.25	
5	0.21		5.14		1.09	

The vitamin C content of the suprarenals does not change, on the other hand the content of the liver undergoes a remarkable increase, under the influence of insulin. This increase may possibly be due to an increased deposition of the vitamin in the liver at the expense of the content of other organs or to an increased synthesis by the liver, but, as it is not likely that the organs would contribute sufficient to cause this considerable rise in the content of the liver and at the same time keep the amount excreted in urine at a maximum, and further as it is found that there is no contribution by the suprarenal, one of the organs which contain the vitamin, the view of an increased synthesis is favoured.

As regards the vitamin C content of the urine, the limits of excretion after insulin administration and fasting for 24 hours are within the limits of excretion of animals simply fasted for 24 hours or just a little less.

The effect of insulin was also tried on rats while diet was given. Each pair of rats was placed in a metabolism cage and the scorbutic diet was provided. The amount of vitamin excreted by these particular animals in 24 hours before the administration of insulin was determined. No abnormality was again noticed. Each rat was then daily injected with 4 units of insulin for 4 consecutive days, the diet being still given. The vitamin excreted was determined daily and on the 5th day the rats were killed for the determination of the vitamin in the suprarenals and livers.

The vitamin contents of four suprarenals and two livers were 0.21-0.23 and 3.50-3.58 mg. respectively. The amount excreted per day during the 4 days of insulin injections was 1.00-1.08 mg. per rat. This indicates that there was no change in the content of either the suprarenals or liver but the amount excreted in 24 hours was always within the limits of excretion in the case of animals injected with insulin and fasted for 24 hours. Since the diet is given there is no explanation for the continued high level of excretion other than the assumption of an increased synthesis.

Effect of adrenaline on storage and excretion of vitamin C.

Beznák & Hariss [1934] tried the effect of adrenaline perfusion on the ascorbic acid content of the left suprarenals of cats, whilst the right glands were removed as normal controls. They found in three out of four cases that there was a decrease of about 20 % of ascorbic acid in the perfused glands, but since in one case a decrease of the same order of magnitude was found in the right suprarenal, they came to the conclusion that perfusion with adrenaline caused no change in the ascorbic acid content. Their conclusion is contradictory to the finding of Euler [1933] that adrenaline injections cause the ascorbic acid to be liberated into the blood. Perfusion of the liver of guinea-pigs, previously saturated with ascorbic acid, also caused no change in the content of these organs.

In the present investigation, rats which had been kept on the scorbutic diet for about 2½ months were placed, in pairs, in metabolism cages. All rats were of the same age and of a weight 275–280 g. They were given the scorbutic diet and the amount of the vitamin was determined in the 24 hours' urine. No abnormality in the vitamin excretion by the particular animals used for the experiments was found. Each rat was then injected with 0.1 ml. of 1/1000 adrenaline hydrochloride solution and no diet was given for the next 24 hours. At the close of that period the pair of rats was killed and the vitamin C contents of the four suprarenals, of the two livers and of the 24 hours' urine collected were determined (Table V).

It can be concluded that adrenaline does not alter the vitamin C content of the suprarenals or of the liver. As regards the amount excreted in urine it can be seen that the administration of adrenaline when the diet is withheld for the 24 hours following the injection causes a remarkable fall below the limits of excretion of similar animals when simply fasted for 24 hours.

Table V.

Exp.	Vitamin in 4 suprarenals mg.		Vitamin in two livers mg.		Vitamin excreted per rat in 24 hours mg.	
	Adrenaline	No adrenaline	Adrenaline	No adrenaline	Fasting and adrenaline	Fasting and no adrenaline
1	0.23		3.50		0.38	
2	0.22		3.72		0.44	
3	0.20	0.20–0.24	3.69	3.29–3.72	0.60	1.10–1.35
4	0.21		3.67		0.52	
5	0.22		3.71		0.36	

Adrenaline is well known to cause glycogenolysis, hyperglycaemia and glycosuria, but it was repeatedly noticed that the urine of rats previously kept for 2½–3 months on the scorbutic diet does not contain sugar after the adrenaline injections. At first it was thought that either the dose was not sufficiently high to cause glycosuria or the preparations of adrenaline used were not sufficiently potent. These possibilities were eliminated by taking a number of rats of practically the same weight, 275–280 g., some of which had been previously kept on the scorbutic diet for the period above mentioned and some on the ordinary diet. Each rat was injected with 0.1 ml. of the same 1/1000 adrenaline hydrochloride solution, and no diet was given after the injections. Samples of 24 hours' urine were separately collected from each rat. It was again found that the urines of rats previously kept on the ordinary diet gave, with no exception, a strong reduction with Fehling's solution, whilst the urines of those kept on the scorbutic diet gave no reduction at all in the majority of cases, or a very slight

reduction only in few cases. The occurrence of this very slight reduction may, at least partly, be ascribed to the possibility that some of the rats had a portion of the diet a shorter time before the injections than others, since it became much less frequent when the following procedure for feeding was adopted. On the morning of one day no diet was given to both batches of animals. The corresponding diets were given for 1 hour from 3 to 4 p.m. on the same day in order to insure simultaneous feeding of all the animals. On the second morning the rats were injected with adrenaline and were left with no food for the 24 hours following the injections. Under these conditions the urines of rats previously kept on the ordinary diet still showed considerable reduction of Fehling's solution after the adrenaline administration.

It was thought that a smaller store of carbohydrate in the body of animals previously kept on the scorbutic diet might be a factor in determining the difference in behaviour. It has been found, however, that the liver glycogen of both sets of animals was of the same order of magnitude at the close of the period usually preceding the adrenaline injections.

A difference which the two different diets might produce in the role of the adrenaline of the body was thought of as another possibility which might determine the difference between the behaviours of animals towards the injected adrenaline. If this is the case, the injection of equal amounts of adrenaline into animals which are otherwise under the same conditions but upon the different diets will cause a difference in the resultant total adrenaline which leads to a difference in the extent of glycogenolysis and of glycosuria in the two cases.

It may be remarked that the amount of adrenaline injected into rats in the two cases is about one and a half times as much as the normal total load of adrenaline of the two suprarenals and therefore a slight difference in the body adrenaline would not account for the marked glycosuria occurring in the case of animals previously kept on the ordinary diet and not in the others after the adrenaline injection. Nevertheless the adrenaline (mg. per g.) of the two suprarenals of rats kept on the scorbutic diet for 2½–3 months and of those kept on the ordinary diet was determined and was found to be the same; 1.890 mg. for those previously kept on the scorbutic diet and 1.892 mg. for those previously kept on the ordinary diet.

The following experiments prove beyond doubt that the difference in behaviour towards adrenaline is determined by the sort of diet given before the injections and is due to vitamin C in particular provided that the other experimental conditions are the same. Four groups of rats of the same age and weight were chosen. The first three were previously kept on the scorbutic diet for the period indicated, the fourth was kept on the ordinary diet. All continued to receive the respective diets. Group II was injected with 7 mg. of ascorbic acid per rat per day for 3 days before the adrenaline injections. Group III was treated like II but the ascorbic acid was added to the diet. On the day before the adrenaline injections the conditions for feeding already described were followed. All groups were injected with adrenaline hydrochloride (0.1 mg. per rat of 280 g.). The administration of ascorbic acid was stopped on the day adrenaline was injected. Examination of the 24 hours' urine following adrenaline injections showed no reduction of Fehling's solution in I, a marked reduction in IV, more reduction in III and a large reduction in II. Further, the reducing substances in terms of glucose in the 24 hours' urine per rat were determined by the method of Shaffer & Hartmann [1920] and the types of urinary osazones were examined under the different conditions (Table VI). This result, firstly, affords further evidence that no glycosuria is caused by adrenaline in the case of group I but a marked

one in the other cases; secondly, that the increased reduction after adrenaline when ascorbic acid had been injected or given by mouth is due to glycosuria and not simply due to the ascorbic acid itself being excreted in the urine; thirdly, that ascorbic acid administration is not capable by itself of producing glycosuria.

Table VI.

Group	Glucose equivalent of reduction in urine (mg.)		Type of urinary osazone	
	Before adrenaline	After adrenaline	Before adrenaline	After adrenaline
I	9-12	10-14	N.M.	N.M.
II	11-16*	53-60	"	N.M. and T.G.C. + + +
III	10-14*	37-44	"	N.M. and T.G.C. + +
IV	11-15	24-29	"	N.M. and T.G.C. +

* After ascorbic acid administration.

N.M. = normal mixture. T.G.C. = typical glucosazone crystals.

The results show that, under the same conditions, equal amounts of ascorbic acid given by mouth and by injections cause, after the administration of adrenaline, less glycosuria in the first than in the second case. This is probably due to destruction or loss of the vitamin in the alimentary canal.

With constant conditions of feeding before the adrenaline administration, the glycogenolysis and the resultant hyperglycaemia and glycosuria caused by adrenaline are, therefore, determined by the ratio of the dose of adrenaline to the body weight and by the degree of saturation of the body with vitamin C. The last statement will be referred to in the discussion.

The next question to investigate was whether the adrenaline, when injected into rats previously kept on the scorbutic diet for $2\frac{1}{2}$ -3 months, causes no mobilization of glycogen and hence no glycosuria or actually causes the former but at a lower rate than in the case of animals previously kept on the ordinary diet. For this purpose, the time at which the maximum rise in blood sugar usually takes place was determined when adrenaline was given in proportion to the body weight and the restrictions previously described were followed. It was found that, in the case of group IV, the maximum rise took place after 1 hour regardless of the body weight of the animals. Groups of rats were chosen and treated as described in the previous experiment. One rat of each group was used for determining the initial blood sugar level and the second received an adrenaline injection proportional to its body weight. All rats were slaughtered 1 hour after the injections and the blood sugar was determined. The mean of the results of a number of experiments is given in Table VII.

Table VII.

Diet	Initial blood sugar g. per 100 ml.	Blood sugar 1 hour after adrenaline g. per 100 ml.
Scorbutic only	0.102	0.210
Ordinary	0.104	0.247
Scorbutic <i>plus</i> ascorbic acid injections	0.112	0.268

While these results give further support to the previous observations they definitely show that adrenaline in all cases causes mobilization of the glycogen store of the body but the rate of mobilization is increased by increasing the saturation of the body with vitamin C.

DISCUSSION.

The discovery of the influence of vitamin C on the rate of mobilization of glycogen when adrenaline is administered is of great importance in making clear some of the results experimentally attained.

The saturation of the body by injecting vitamin C markedly increases the rate of glycogenolysis caused by adrenaline; further, the results show that this rate runs parallel with the degree of saturation of the body with that vitamin. Thus the highest rate for the mobilization of glycogen is attained by producing the highest saturation by means of injecting the vitamin, for 3 days, into rats previously kept on a diet deficient in that vitamin for $2\frac{1}{2}$ –3 months; a lower rate by adding the pure vitamin to the deficient diet of similar rats in similar amounts and for similar periods, when part of the amount given may be supposed to undergo destruction or loss; a still slightly lower rate by keeping similar rats on an ordinary mixed diet containing the vitamin; and the lowest rate when no vitamin is externally provided for the period above mentioned. This necessitates the assumption that, although the synthetic process is generally enhanced after the 9th day on the scorbutic diet and the level of excretion in urine also gradually rises and reaches certain higher limits from the 16th day onwards, yet the rats are to be regarded as in a less saturated condition than when they are kept on the ordinary diet. In fact, as has already been pointed out, the level of excretion of the vitamin in the urine from the 16th day onwards on the scorbutic diet is generally lower than the level of excretion when the animals are kept on the ordinary diet. These statements are not contradictory to the fact that these animals do not develop scurvy when kept on a scorbutic diet, since the amount of vitamin synthesized may be sufficient to ensure good health, while producing a relatively lower saturation than when the animals are kept on the ordinary diet. A similar finding, in the case of man, has been reported by Johnson and Zilva [1934], who found that it is possible to exist on diets containing sufficient vitamin C to insure freedom from scurvy even when the store of the vitamin in the body is maintained low.

It is indicated above that the rate of mobilization of glycogen under the influence of adrenaline runs parallel with the degree of saturation of the body with vitamin C. This rate can also be influenced by different factors. Since the adrenaline is given in proportion to the body weight and variable factors, such as the conditions of feeding before the administration of adrenaline and the age of rats, are controlled, whilst other factors, such as the adrenaline and glycogen contents of the suprarenals and liver respectively, are constant both in animals kept on the scorbutic diet for $2\frac{1}{2}$ –3 months and in those kept on the ordinary diet, it becomes obvious that the degree of saturation of the body with vitamin C is a direct factor in determining the rate of glycogenolysis. Against this statement must be set the fact that saturation of the body with ascorbic acid alone has been proved in the present work to produce no hyperglycaemia or glycosuria; but the rate of rise of blood sugar is determined by the rates of the mobilization of glycogen and of the utilization of the blood sugar and unless the latter is interfered with, as is done by injecting adrenaline, the effect of the degree of saturation of the body with the vitamin on the rate of rise of blood sugar will be rendered inconspicuous. This becomes more evident if we take into consideration the recent work of Strieck [1935], who found that vitamin C, administered in different manners, increases the oxygen consumption without affecting the respiratory quotient or, in other words, that the carbohydrates are included in the substances utilized for the rise of metabolism caused by the vitamin itself.

That adrenaline decreases the utilization of blood sugar in the peripheral tissues has been indicated by Cori & Cori [1928], by Sahyun & Luck [1929], and by Cori *et al.* [1930].

An alternative explanation for the parallelism between the rate of glycogen mobilization and the degree of saturation of the body with vitamin C under the influence of adrenaline is the possibility that the vitamin in the reduced form protects the adrenaline from rapid oxidation and so, by increasing the saturation of the body with the former, a certain dose of the latter will be rendered more effective in producing the glycogenolysis. Such a relationship between the two substances in the case of the suprarenals has been suggested by different investigators owing to the co-existence of the two substances in the same organ and to the reduction in the amount of both stated by some to occur in scurvy. According to Deutsch & Schlapp [1935], however, there does not appear to be any close relationship between the two substances. Further, the finding of Johnson & Zilva [1934] that the oxidized form of ascorbic acid is not excreted in the urine even after the ingestion of ascorbic acid in that form may at present stand against the latter explanation.

Considering, as a whole, the results obtained when trying the effects of starvation for 24 hours, of insulin and starvation, of insulin and feeding and of adrenaline and starvation, it is found that the apparent variations in these four cases can be explained on the assumption that the vitamin is utilizable in some way during the metabolism of the different substances, so that there is more utilization whenever there is rise in metabolism and less utilization when the metabolism is relatively lowered. Thus, in the case of starvation for 24 hours when the metabolism is relatively lower than when the animals are fed the utilization of the vitamin becomes less and the amount saved causes a rise in the vitamin excretion in the urine. After adrenaline administration, a rise in metabolism was indicated by Sandiford [1920] and by Cori & Cori [1928] and at the same time there is no evidence of increased synthesis of the vitamin; therefore, the increased utilization of the latter accompanying the rise in metabolism results in a remarkable fall in the amount excreted in the urine. In the two cases of insulin and starvation for 24 hours and insulin and feeding the metabolism is raised but there is more continual rise in the second case than in the first owing to the continual supply of metabolites; at the same time there is evidence of increased synthesis of the vitamin under the influence of insulin, consequently the excess in the utilization of the vitamin is supplied by its synthesis. In the first case the synthesis exceeds the utilization resulting, therefore, in a rise of the vitamin in the liver, whilst in the second case the vitamin synthesized in the liver under the influence of insulin does not accumulate in that organ but is transferred to the tissues to meet the requirements of the increased metabolism. In spite of the fact that there is a rise in metabolism, the level of excretion of the vitamin in the urine is high in both cases, which is contrary to the case of adrenaline, and may thus indicate a continually raised charge of the blood by the synthesized vitamin.

SUMMARY.

1. The total vitamin C content of the suprarenals and of the livers of adult rats of initial body weight 220–230 g. is constant whether the animals are provided with an ordinary or with a scorbutic diet.

On a scorbutic diet, rat's suprarenals show an increase in size and in weight which is noticeable only for a certain period.

2. When the ordinary diet is replaced by a scorbutic one, the level of excretion of vitamin C in the urine of rats rapidly falls and remains low for a certain

period, after which it gradually increases owing to the enhancement of the synthetic process, but remains at a lower level than on the ordinary diet.

3. Starvation for 24 hours does not alter the vitamin C content of the suprarenals or of the livers of rats previously kept on a scorbutic diet, but it causes an increase in the amount excreted in the urine.

4. Injection of insulin into rats previously kept on a scorbutic diet increases the vitamin content of the liver, probably owing to increased synthesis. The content of the suprarenals is not affected.

5. Adrenaline does not alter the vitamin C content of the suprarenals or of the liver of rats previously kept on a scorbutic diet, but it lowers the level of excretion in the urine.

6. The rate of glycogenolysis caused by adrenaline runs parallel with the degree of saturation of the body with vitamin C. It is suggested that this degree of saturation is a direct factor in determining the rate of glycogenolysis, or that the vitamin in the reduced form protects the adrenaline from rapid oxidation.

7. The vitamin synthesis by rats kept on a scorbutic diet may be sufficient to insure freedom from scurvy while producing a relatively lower saturation of the body than when the animals are kept on a diet containing the vitamin.

8. It is suggested that vitamin C is utilized in some way during the metabolism of the different substances, so that there is a parallelism between the extents of the two processes.

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