SUMMARY

1. Antrycide has been determined by reaction with eosin to give a highly fluorescent salt which can be separated from excess eosin by extraction into a mixture of chloroform and butanol.

2. The method measures down to 40 $\mu\text{g.}$ of Antrycide/l. of plasma with satisfactory accuracy, and 20 $\mu\text{g./l.}$ approximately.

3. The method has a considerable degree of specificity, in that most other tertiary and quaternary bases examined did not react.

4. It can be applied to tissues and urine as well as plasma.

5. A less sensitive method suitable for plasma involves measuring the weak blue fluorescence of Antrycide itself.

6. Two colorimetric methods, suitable only for aqueous solutions, are briefly described.

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The Influence of Scurvy and Fall in Weight in Young Guinea Pigs on the Alkaline Phosphatase Content of the Serum and the Zones of Provisional Calcification

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Some years ago Robison and his collaborators (Robison, 1932), as a result of a series of brilliantly conceived and interpreted investigations, broke new ground by advancing some hypotheses on the function of phosphatases in the animal body, particularly in the process of ossification. These contributions led to an extension of the field of inquiry, thus attracting the attention of the clinical pathologist. In their pioneer work, Smith & Maizels (1932) and Smith (1933) found, amongst other observations, that the plasma phosphatase of infants and young children suffering from acute scurvy was low, and that the enzyme concentration in the plasma rose on treatment. Similar observations were made by Scöz, Cattaneo & Gabbielli (1937), Todhunter & Brewer (1940) and Shwachman and Gould (Shwachman, 1941; Gould & Shwachman, 1941, 1943; Shwachman & Gould, 1942) on young guinea pigs

suffering from scurvy. Scurvy, however, is not the only condition responsible for a diminution in the phosphatase of the blood. The early experiments of Smith (1933) have already shown that several diseases associated with arrested growth, or with severe marasmus, such as coeliac disease, achondroplasia and cretinism, also produce such a fall in the blood phosphatase. Similarly, in prolonged arrested growth and in loss of weight a striking drop in the serum phosphatase can be observed in young guinea pigs (Watkins & Zilva, unpublished results).

In growing guinea pigs maintained on a scorbutic diet the development of scurvy and fall in weight overlap, and it is therefore not easy to ascertain with any degree of certainty whether the observed fall in serum phosphatase is due to one or other of these causes. Todhunter & Brewer (1940), in controlling their experiments, used the 'paired-feeding tech-

nique' in which the food intake was controlled but not the growth of the animals. In fact their experiments showed that the scorbutic animals lost considerably more weight than their paired mates, and consequently the diminution in the serum phosphatase of the scorbutic animals might have been traced to either of the above-mentioned causes. Nor did Scoz *et al.* or Shwachman and Gould take the change in weight of the experimental animals into consideration. The object of the present investigation was to ascertain beyond doubt whether scurvy *per se* is responsible for the fall of the phosphatase in the serum and in the zones of provisional calcification of the costochondral junctions and the proximal ends of the tibiae of growing guinea pigs and, if so, to what extent the almost simultaneous cessation of growth and the loss in weight influence this change in the enzyme activity of the tissues in question. As might be expected in an inquiry of this nature, indications of other phenomena were obtained which went beyond the original object of the work and which call for further investigation.

METHODS

Experimental animals and diet. The growing guinea pigs employed in this investigation were about 6-8 weeks old, ranging in weight from 280 to 330 g. The individual groups were arranged so that the average weight was usually about 300 g. The weight of the adult guinea pigs ranged from 700 to 900 g. The vitamin C-deficient diet was that described by Penney & Zilva (1946). The mixed diet consisted of one part of oats and two parts of bran by volume, with cabbage and water *ad lib.* Only young animals which showed normal growth during a preliminary period of 7 days preceding the experiment were employed. On our scorbutic diet some of the growing guinea pigs showed macroscopic lesions such as petechiae after about 11 or 12 days. After 15 days on the diet marked haemorrhages were found in most animals and death from scurvy occurred after about 25 days. The animals consumed the scorbutic diet fairly readily even from the first day, although there was often little increase in weight during the first day or two. This was probably due to the disappearance from the caecum of the cabbage consumed during the preliminary period and not to a genuine stoppage of growth which could bring about a fall in the tissue phosphatase.

In order to induce a desired drop in weight within a certain time the animals were weighed daily and the quantity of the basal diet offered on any particular day was determined by the loss in weight incurred during the preceding 24 hr.

Preparation of tissues. The guinea pigs were killed by stunning and bleeding. The blood, which was collected in test tubes of about 25 mm. diameter, was allowed to clot at room temperature for about 3 hr. After loosening the clot with a glass rod the contents of the tubes were maintained at 3° overnight. The serum was then decanted from the clot and centrifuged. Phosphatase determinations were carried out on the cell-free serum, when possible the same day. When it was not possible to do so the serum was stored at -20°, never longer than 14 days, during which time the enzyme was found by us not to deteriorate.

After post-mortem examination all the necessary bones were thoroughly freed of adhering tissue. In the case of the costochondral junctions pieces consisting of 1 mm. of the cartilage and of the bone on each side of the junctions of all the ribs were removed and pooled together for extraction. Although experiments with mixed diet animals have shown little variation in the phosphatase content from rib to rib, it was considered that any change in the enzyme content which might occur from group to group would be indicated more accurately if the phosphatase value of the pooled rib junctions of each animal were obtained. The epiphyses with the adjoining epiphyseal cartilage from the proximal end of both tibiae, which will be referred to in this communication as the epiphyses, were similarly pooled. We found that the water content and ash of the above tissues did not change significantly during the development of scurvy or after a fall in weight produced by a quantitative restriction of the basal diet. The enzyme from the costochondral junctions and the epiphyseal tissues was always extracted with the following convenient volumes of CHCl_3 -saturated water, 40 times the mass of the former and 20 times that of the latter. The extraction was carried out by allowing the bones to stand in the CHCl_3 -saturated water overnight at 3°, grinding the tissues with phosphate-free silver sand, returning them to the CHCl_3 -water and continuing the extraction at 3° for another 24 hr. After centrifugation, the supernatant liquid was used for the determination of phosphatase. It may be mentioned that Gould & Shwachman (1941) utilized, apart from the proliferating cartilage, much more than we did of the adjoining tissues which must have contained lower and probably more variable amounts of the enzyme than the very active ossification centres.

In the extraction of the soft tissues the following quantities of CHCl_3 -saturated water were used: liver, 1 ml./g.; kidney, 4 ml./g.; large intestine, 4 ml./g. and small intestine, 15 ml./g. The entire organs (the contents of the intestines being completely washed out with saline and the tissues dried with filter paper) were thoroughly ground with phosphate-free silver sand, centrifuged and the enzyme determined in the resulting supernatant extract.

Determination of phosphatase activity. The substrate used was sodium β -glycerophosphate, and the hydrolysis was carried out according to the method of Shinowara, Jones & Reinhart (1942). In all determinations the pH of the substrate was adjusted so that the reaction took place at pH 9.3. When the activating effect of Mg was determined, a MgCl_2 solution was added to the stock substrate so that its final concentration in the reaction mixture was 0.009 M. In the case of the serum, owing to the higher pH to which the substrate had to be brought (pH 10.9) in order to achieve the final pH 9.3, the MgCl_2 solution had to be added directly to the reaction mixture in order to prevent precipitation of $\text{Mg}(\text{OH})_2$. The determination of inorganic phosphate present before and after incubation at 37° was carried out by the Briggs method, as modified by Martland & Robison (1926). A photoelectric colorimeter was employed. The phosphatase activity was expressed in terms of mg. P liberated as orthophosphate from the substrate in 1 hr. under the above experimental conditions, in the case of the serum by 100 ml. and in that of the bones, liver, kidneys and intestine by 1 g. of wet tissue. All phosphatase determinations in the serum and the bones were carried out in the presence and in the absence of added Mg. The results throughout were found to be parallel, the addition of Mg increasing the phosphatase

activity by about 30% in the serum and about 70% in the bones. We are therefore omitting the Mg figures.

Determination of L-ascorbic acid. For the preparation of the trichloroacetic acid extracts see Penney & Zilva (1946). Roe & Kuether's (1943) 2:4-dinitrophenylhydrazine method was used for the L-ascorbic acid determination.

RESULTS

Change in the phosphatase content of certain tissues during the development of scurvy in growing guinea pigs

These changes were investigated in the serum, the costochondral junctions and the epiphyses. Groups of animals maintained for 10, 15, 18 and 21 days on the scorbutic diet, offered *ad lib.*, were investigated for the phosphatase content of all the three tissues.

Fig. 1. The phosphatase content of the serum, costochondral junctions and epiphyses of growing guinea pigs on scorbutic diet. Percentage of initial body weight, ●—●—●; phosphatase activity of serum, ○—○; costochondral junctions, □—□ and epiphyses, △—△. Twelve animals were used in each group. Standards of phosphatase activity in this and all subsequent Figures and Tables are given in the experimental section.

In addition, groups of animals killed after 11, 12, 13 and 14 days, respectively, were examined for the phosphatase content of their sera only. Owing to the marked individual variations in the phosphatase contents of the tissues we found in preliminary experiments that when the phosphatase means of a particular group showed a standard deviation greater than 5.0 it was not reliable for comparison, a point to be borne in mind. We usually obtained standard deviations lower than this value when the groups consisted of not less than six animals, and we therefore employed this number of animals per group in subsequent experiments. In this set of experiments, however, owing to its pivotal character in the general investigation, the number of animals was increased to twelve per group. The results (Fig. 1) offer a detailed aspect of the phosphatase content of the tissues in question at the various crucial stages of the development of scurvy up to the premortal phase.

The first point to consider is that when a line is drawn approximately through the points representing the average weights of the different groups we obtain a curve very similar to that given by guinea pigs of this age maintained on our scorbutic diet, namely the animals grow up to about 15 days and then their weights decline rapidly. Furthermore, we may mention that the post-mortem lesions of the groups at the various times were characteristic of the development of scurvy in animals of such initial weight. Mild macroscopic lesions such as petechiae were first observed in the vicinity of the knee joints in a few of the animals of the group after 11 days. The intensity of the haemorrhages and the number of animals affected in the group then increased with each day. After 15 days on the diet marked haemorrhages were found in most of the animals. The severity of the scorbutic signs then increased until the premortal phase. A typical picture of the development of scurvy is obtained from these composite results, and it may be safely inferred that in all probability the changes in the phosphatase content of the bones were equally representative.

It is evident that a striking drop in the average phosphatase concentration of the serum took place between the eleventh and twelfth days when the first macroscopic signs of scurvy began to appear in some of the animals of these groups, but when, it must be noted, all the animals were nevertheless growing well. This decline in the serum phosphatase concentration continued until the premortal phase was reached. The freely drawn lines in the figure between points obtained in the bone analyses may be at first somewhat misleading and need comment. In the case of the costochondral junctions the difference between the points obtained after 10 and 15 days is not statistically significant by Student's *t* test; nor is the difference between the points at the beginning of the experiment, and after 10 and 15 days statistically significant in the case of the epiphyses. In both the bone tissues, unlike in the serum, the statistically significant decline in the phosphatase content began more or less simultaneously with the fall in weight in the respective groups and then, as in the serum, it became rapid. The phosphatase value for costochondral junction after 21 days does not indicate a true slowing down in the fall of the enzyme content in scurvy at the premortal phase, because it was not found to be reproducible in subsequent experiments.

Gould & Shwachman (1941), by comparing the phosphatase contents of the serum, the costochondral junctions and the tibia of guinea pigs maintained on a scorbutic diet for 21 days with those of animals receiving a 'vegetable diet', concluded that 'the fall in bone phosphatase activity parallels the fall in serum phosphatase activity'. Our results

show that such parallelism does not exist, since the serum phosphatase began to fall several days before that of the bones, and we are of the opinion that had Gould & Shwachman investigated the intermediate stages in the development of scurvy they would have arrived at a conclusion similar to ours.

The influence of change of weight on the phosphatase content of certain tissues of young guinea pigs

This problem was investigated in three sets of experiments.

(1) In one set the phosphatase content of the three tissues previously investigated was established in one group of guinea pigs which subsisted on a restricted scorbutic diet supplemented with a daily dose of 25 mg. L-ascorbic acid, so that a drop in weight of about 20% was accomplished in 9 days. Another group was similarly restricted for 9 days, but offered a mixed diet *ad lib.* for a further 6 days, and a third group after maintenance for 9 days on restricted diet was offered a mixed diet *ad lib.* for 12 days (Fig. 2).

Fig. 2. The phosphatase content of the serum, costochondral junctions and epiphyses of young guinea pigs after a fall in weight during the first 9 days induced by quantitative restriction of the diet, and a subsequent resumption of growth caused by the consumption of a mixed diet with cabbage *ad lib.* These animals received a daily dose of 25 mg. of L-ascorbic acid in addition to the scorbutic diet during the first 9 days. Percentage of initial body weight, ●---●; phosphatase activity of serum, ○---○; costochondral junctions, □---□ and epiphyses, △---△. Six animals were used in each group.

(2) All groups in this set were maintained on a scorbutic diet without any supplement of vitamin C, and were restricted in their food intake in the first place for 9 days, so that a loss in weight of about 23% was incurred, after which time the basal diet was offered *ad lib.* to the remaining groups. The following groups were investigated in this set of experiments: after 9 days restriction only, and after 9 days restriction followed by 6, 9 and 12 days, respectively, on scorbutic diet *ad lib.*, i.e. after 15, 18

and 21 days from the beginning of the experiment (Fig. 3).

(3) All the animals in this set were maintained on the scorbutic diet which was supplemented with a daily dose of 25 mg. L-ascorbic acid. The daily intake

Fig. 3. The phosphatase content of the serum, costochondral junctions and epiphyses of young guinea pigs on a scorbutic diet without a supplement of L-ascorbic acid after a fall in weight during the first 9 days induced by quantitative restriction of the diet, and subsequent resumption of growth caused by *ad lib.* consumption of the diet. Percentage of initial body weight, ●---●; phosphatase activity of serum, ○---○; costochondral junctions, □---□ and epiphyses, △---△. Six animals were used in each group.

Fig. 4. The phosphatase content of the serum, costochondral junctions and epiphyses of young guinea pigs on a scorbutic diet after a fall in weight during the first 9 days induced by quantitative restriction of the diet. The growth was subsequently maintained on a level similar to that which prevailed among the animals portrayed in Fig. 3, by controlling the intake of the basal diet. 25 mg. of L-ascorbic acid was administered daily throughout this experiment. Percentage of initial body weight, ●---●; phosphatase activity of serum, ○---○; costochondral junctions, □---□ and epiphyses, △---△. Six animals were used in each group.

of food was, however, regulated in such a way that the weights of the respective groups investigated at the various times were similar to those in the preceding set, the main difference being that all the animals in the third set (Fig. 4) were protected from scurvy.

From Fig. 2 it will be seen that after a drop in weight of 20 % in 9 days the phosphatase fell by 58, 53 and 50 % in the serum, costochondral junctions and epiphyses respectively. On offering a mixed diet *ad lib.* at this stage the animals resumed rapid growth, attaining approximately their original weight after 6 days, and continued their growth at a considerable rate even after that time. The phosphatase values, on the other hand, had already reached the normal figures after 6 days on the mixed diet *ad lib.*, with the exception of the costochondral junctions ($t=3.82$, $P<0.01$). After 12 days on this diet no differentiation from the normal could be found in any of the tissues (serum, $t=0.62$, $P=0.5-0.8$; costochondral junctions, $t=1.72$, $P=0.1-0.2$; epiphyses, $t=0.87$, $P=0.4-0.5$). The maximum phosphatase was thus reached within a very few days after resumption of growth when the growth was rapid.

When the development of scurvy was not prevented by the administration of L-ascorbic acid the resumption of growth after 9 days on a restricted diet followed a different course. For example, in the second set of experiments (Fig. 3), the groups of animals, which owing to restriction of diet had lost about 23 % of their original weight in 9 days, offered the scorbutic diet *ad lib.* resumed growth at a somewhat slower rate than in the preceding set and after 9 days reached the maximum, which was followed by a fall in weight. This fall was due to the onset of scurvy. In this set of experiments the 23 % fall in weight after 9 days' restriction brought about a fall in the phosphatase contents as follows: serum, 42 %; costochondral junctions, 41 %; and epiphyses, 27 %. After 6 days of growth on the unrestricted basal diet *ad lib.* no significant difference in the phosphatase contents of these three tissues was observed in the second group of guinea pigs (serum, $t=0.88$, $P=0.3-0.4$; costochondral junctions, $t=2.11$, $P=0.05-0.1$; epiphyses, $t=0.51$, $P=0.6-0.7$). After 9 days, when the animals reached their maximum weight, there was a significant fall in the serum value ($t=2.83$, $P=0.01-0.02$), but no change in the values of the other two tissues. After 12 days on the basal diet *ad lib.* the serum and both the bone phosphatase figures were significantly lower than the maximum values reached after the end of the restricted period (serum, $t=6.90$, $P<0.01$; costochondral junctions, $t=4.46$, $P<0.01$; epiphyses, $t=2.64$, $P=0.01-0.02$).

If the general trends of the fall in weight and in phosphatase values in this set of experiments are compared with those observed in the normal development on a scorbutic diet (Fig. 1) it is seen that they are similar, except that all these falls were delayed for a few days. It was therefore interesting to find that the scorbutic lesions observed at autopsy were similarly delayed. Thus the degrees of scurvy found at 15 and 21 days, respectively, on the scorbutic diet

in this set of experiments were equivalent to those observed at 11 and 15 days in the experiments of the preceding section. In this connexion it may be mentioned that although the onset and development of scurvy, the fall in weight and the fall in the phosphatase contents were delayed in the second set of experiments as compared with the animals which received the scorbutic diet *ad lib.* without previous restriction (cf. Fig. 1), the phosphatase contents of the tissues after 21 days in the two sets of experiments are not significantly different.

In the third set of experiments (Fig. 4), the influence of scurvy was eliminated and consequently any change in the phosphatase contents of the tissues was entirely due to changes in growth such as occurred in the animals of the second set. The striking fact that emerges from these experiments is that the guinea pigs, unlike those which were not protected from scurvy, did not show any fall whatsoever in the serum phosphatase content below that observed after 9 days, not even after 21 days. In fact, there was a significant rise after 15 days when resumed growth was still in progress ($t=2.45$, $P=0.02-0.05$). Nor was there such a fall in the phosphatase content in either of the bones after 21 days, in spite of the fact that there was a drop in weight of the animals in this group after 17 days. It may therefore be concluded that the diminution in the phosphatase content observed in the second set of experiments was due to the development of scurvy and not to the fall of weight.

The influence of scurvy and change of weight on the phosphatase content of certain tissues in adult guinea pigs

In the preceding experiments young growing guinea pigs in which the osteoblastic activity was high were employed. In fully grown animals, in which erosion and redeposition of the calcified areas are more or less in equilibrium, this activity is very much diminished. It was therefore of interest to investigate the phosphatase content in the three tissues in adult guinea pigs: (1) maintained on a scorbutic diet; (2) maintained on a scorbutic diet with a dose of 25 mg. L-ascorbic acid daily, but restricted in their food intake so as to produce in 25 days a fall of about 20 % of the original weight; and (3) maintained on the same diet for 20 days, restricted in their food intake so as to bring about a similar fall in weight during this time, and then offered a mixed diet *ad lib.* for a further 5 days in order to induce growth. The results are represented graphically in Fig. 5. The serum, costochondral junctions and the tibiae (parts of the bones identical with those of the growing guinea pigs were taken for analysis) from two groups of animals, which had subsisted on the scorbutic diet for 23 and 28 days respectively, were analysed for their phosphatase

content. In an additional group the serum only was analysed after 10 days on the scorbutic diet. In the other two experiments all the tissues were analysed after 25 days. It is seen that there was a significant drop after 23 and 28 days in the phosphatase content

the knee joints and in most of the costochondral junctions. On the other hand, in the animals protected from scurvy but maintained on the restricted scorbutic diet there was not any significant fall in the phosphatase content of the serum or the tibia. The phosphatase values of the costochondral junctions, however, both in the group which was restricted for 25 days and in the one which was restricted for 20 days and then allowed to grow freely for 5 days, were significantly lower than the corresponding values of the animals on the mixed diet (first group, $t=2.42$, $P=0.02-0.05$; second group, $t=2.27$, $P=0.02-0.05$). Whether this fall has any physiological significance is not easy to judge from this limited number of experiments. It is, however, clear that in the bones of these adult animals in which the osteoblastic activity was low, scurvy did not change the phosphatase content significantly, whilst in the serum it was responsible for a definite diminution in the phosphatase concentration. This fall was, however, very much smaller than that observed in the case of young animals.

The phosphatase activity of some tissues of growing guinea pigs not saturated with L-ascorbic acid

For the complete understanding of the preceding experiments it was necessary to establish the effect of the degree of saturation with L-ascorbic acid on the phosphatase content of the tissues of growing guinea pigs fully protected from scurvy. A daily dose of 2 mg. L-ascorbic acid to growing guinea pigs on the scorbutic diet protects these animals from macroscopic, and in all probability also from microscopic, lesions of scurvy, although the L-ascorbic acid content of the tissues of such animals is of a very low order. On the other hand, the daily administration of 25 mg. of L-ascorbic acid ensures the utmost concentration in the tissues of the animals. Two groups of animals receiving the scorbutic diet and a daily dose of 2 and 25 mg. L-ascorbic acid, respectively, were therefore maintained for 29 days. The average weight of the former group rose from 276 to 370 g. (34 %) and of the latter from 278 to 365 g. (31 %). It will be seen from Table 1 that the phosphatase contents of the three

Fig. 5. The phosphatase content of the serum, costochondral junctions and epiphyses of adult guinea pigs on scorbutic and quantitatively restricted diets. I, scorbutic diet *ad lib.* II, quantitatively restricted scorbutic diet, with 25 mg. of L-ascorbic acid daily. III, quantitatively restricted scorbutic diet with 25 mg. of L-ascorbic acid daily for 20 days and then a mixed diet with cabbage *ad lib.* for a further 5 days. Average weights of respective groups when sacrificed, ● - - - ●; the interrupted line in II and III represents the trend of the daily average weights of the animals in these groups. Phosphatase activity of serum, ○—○; costochondral junctions, □—□ and epiphyses, △—△. Six animals were used in each group.

of the serum, but not of the bones, in the groups maintained on the scorbutic diet (23 days, serum, $t=3.90$, $P<0.01$. 28 days, serum, $t=3.74$, $P<0.01$; costochondral junctions $t=1.69$, $P=0.1-0.2$; tibiae, $t=0.67$, $P=0.5-0.6$). In both these groups all the animals showed small haemorrhages in the region of

Table 1. *The phosphatase content of the tissues of growing guinea pigs not saturated with L-ascorbic acid*

(For standards of activity see section on Methods)

No. of animals	Diet		Serum	Costochondral junctions	Epiphyses
			(mg. P liberated/ 100 ml./hr.)	(mg. P liberated/ g. tissue/hr.)	(mg. P liberated/ g. tissue/hr.)
12	Mixed diet	Mean	15.9	18.2	12.0
		S.D.	4.3	4.4	4.6
6	Scorbutic diet + 25 mg. L-ascorbic acid daily for 29 days	Mean	16.9	17.2	12.4
		S.D.	3.1	3.0	1.5
6	Scorbutic diet + 2 mg. L-ascorbic acid daily for 29 days	Mean	19.4	15.7	11.1
		S.D.	3.0	2.7	3.2

tissues in the unsaturated guinea pigs did not differ significantly from those of the fully saturated groups maintained either on the basal diet and a daily dose of 25 mg. of L-ascorbic acid or on a mixed diet with cabbage *ad lib.* The concentrations of the phosphatase in the above tissues is therefore not influenced by high concentrations of L-ascorbic acid in the tissues of the animals.

The influence of the L-ascorbic acid content of the diet of growing guinea pigs on the phosphatase content of some of the tissues after 10 days on a scorbutic diet

One of us (Zilva, 1936) has shown that when growing guinea pigs received a daily dose of 2-3 mg. of L-ascorbic acid for 10 days and were then deprived of the vitamin, they succumbed to scurvy after a period of time similar to that observed with animals which were previously maintained on a mixed diet with cabbage *ad lib.*, namely 27-33 days. Smaller daily doses of L-ascorbic acid during the preliminary 10 days brought about earlier death from scurvy. The scorbutic diet in these experiments was similar to that employed in this investigation with the exception that the milk prepared from a dry powder was inactivated by aeration of a hot solution of the reconstituted milk. During the war it was observed that this treatment was not sufficient to inactivate to the usual extent the milk from a commercial powder then supplied (cf. Penney & Zilva, 1945), and in consequence since then recourse was had in this laboratory to the use of copper as a catalyst. This treatment of the reconstituted milk brought about a somewhat more drastic inactivation, and in consequence the experimental animals succumbed on the basal diet after 22-29 days instead of 27-33 days; it was further found that a preliminary daily administration of 2 mg. of L-ascorbic acid was sometimes inadequate to prevent animals from succumbing to scurvy rather earlier than when a mixed diet with cabbage *ad lib.* was offered in the pre-experimental period. By trial and error it was found that this difference could be offset in the new diet by the administration of a daily dose of 0.05 mg. L-ascorbic acid during the experimental period on the scorbutic diet.

Preliminary experiments showed that the influence of the L-ascorbic acid content of the diet, preceding the 10 days of vitamin C deprivation, on the phosphatase content of serum, costochondral junctions and epiphyses was not always in consonance with that exercised on the time taken for the experimental animals to succumb to scurvy. Thus guinea pigs which received certain doses of L-ascorbic acid during the pre-experimental period and which behaved in their development of scurvy in a way identical with that of animals which previously existed on a mixed diet, nevertheless

differed sometimes from the latter guinea pigs in their phosphatase content after 10 days on a scorbutic diet containing reconstituted milk inactivated by copper catalysis.

In order to obtain a clear picture of this problem it was decided to investigate animals on the present scorbutic diet alone and on the scorbutic diet with an addition of 0.05 mg. of L-ascorbic acid daily. The results are incorporated in Tables 2 and 3. It will be seen that the animals which received 2 mg. L-ascorbic acid in the pre-experimental period (group B) and which, as already mentioned, succumb to scurvy earlier than those (group A) which received a mixed diet and cabbage *ad lib.* previous to being placed on the scorbutic diet, showed, after 10 days on the scorbutic diet, a significantly lower phosphatase content in all the three tissues than the animals in group A. At the same time it will be noted that, as might have been expected, the former group showed haemorrhages at the post-mortem examination. When 10, 15 and 25 mg. of L-ascorbic acid respectively were given during the pre-experimental period (groups C, D and E) the phosphatase content rose significantly in the serum and the epiphyses but not in the costochondral junctions. All these figures were nevertheless lower than those obtained in group A, in spite of the fact that no scorbutic lesions were found at the post-mortem examination and that the time taken by such animals to succumb to scurvy was the same as if they had received a mixed diet with cabbage *ad lib.* in the pre-experimental period. Doses above 10 mg. L-ascorbic acid in the preliminary period did not further increase the phosphatase content after 10 days on a scorbutic diet.

When, however, the present scorbutic diet was modified by the addition of a daily dose of 0.05 mg. L-ascorbic acid, so as to simulate the prewar scorbutic diet used in this laboratory, the phosphatase contents of the three tissues of the animals which received 10 mg. L-ascorbic acid daily in the pre-experimental period (group C') reached the same values as those obtained with group A; with 2 mg. L-ascorbic acid during the preliminary period, the administration of 0.05 mg. daily in the experimental period (group B') raised the phosphatase content in the epiphyses only, and then not to the same value as in group A. It will be noted that in this group macroscopic signs of scurvy were found at autopsy. This may not always occur, since 2 mg. L-ascorbic acid in the pre-experimental stage under these conditions is a borderline dose. Unfortunately no intermediate doses in the pre-experimental period between 2 and 10 mg. were tried. It is, however, striking that the consumption of a very small quantity such as 0.05 mg. L-ascorbic acid daily should exercise such an influence on the phosphatase content of all the three tissues after 10 days on the

Table 2. *Phosphatase content of the tissues of guinea pigs after 10 days on scorbutic diet, which have previously received varying quantities of L-ascorbic acid*

Group	No. of animals	Vitamin C consumption during the pre-experimental period of 14 days	Diet during the experimental period of 10 days	Serum (mg. P liberated/100 ml./hr.)			Costo-chondral junctions (mg. P liberated/g. tissue/hr.)		Result of post-mortem examination
				Mean	S.D.		Mean	S.D.	
A	12	Mixed diet	Scorbutic diet	16.2	3.6		18.3	2.7	No macroscopic scorbutic lesions
B	6	Scorbutic diet + 2 mg. of L-ascorbic acid daily	Scorbutic diet	6.6	3.0		10.8	3.6	Slight to moderate macroscopic scorbutic lesions
C	6	Scorbutic diet + 10 mg. of L-ascorbic acid daily	Scorbutic diet	11.6	4.4		11.8	3.0	No macroscopic scorbutic lesions
D	6	Scorbutic diet + 15 mg. of L-ascorbic acid daily	Scorbutic diet	10.7	2.3		13.5	1.0	No macroscopic scorbutic lesions
E	6	Scorbutic diet + 25 mg. of L-ascorbic acid daily	Scorbutic diet	10.3	1.4		11.4	2.3	No macroscopic scorbutic lesions
B'	6	Scorbutic diet + 2 mg. of L-ascorbic acid daily	Scorbutic diet + 0.05 mg. of L-ascorbic acid daily	6.1	2.0		11.7	2.3	Macroscopic scorbutic lesions somewhat less than in group B
C'	4	Scorbutic diet + 10 mg. of L-ascorbic acid daily	Scorbutic diet + 0.05 mg. of L-ascorbic acid daily	13.1	1.7		16.9	1.9	No macroscopic scorbutic lesions

Table 3. *Statistical analysis of the results given in Table 2*

Tissue	<i>t</i>	<i>P</i>	Groups compared									
			AB	AC	AD	AE	AB'	AC'	BC	BD	BE	CC'
Serum			5.60*	2.39*	3.38*	3.84*	6.35*	1.63	2.31*	2.65*	2.74*	0.64
			<0.01	0.02-0.05	<0.01	<0.01	<0.01	0.1-0.2	0.02-0.05	0.02-0.05	0.02-0.05	0.5-0.6
Costochondral junctions			5.00*	4.64*	4.17*	5.30*	6.21*	0.96	0.52	1.76	0.34	2.98*
			<0.01	<0.01	<0.01	<0.01	<0.01	0.3-0.4	0.6-0.7	0.1-0.2	0.7-0.8	0.01-0.02
Epiphyses			4.91*	2.58*	2.13*	2.82*	2.28*	0.90	3.27*	5.49*	3.22*	3.90*
			<0.01	0.02-0.05	0.02-0.05	0.01-0.02	0.02-0.05	0.3-0.4	<0.01	<0.01	<0.01	0.02-0.05

* Denotes a significant difference.

scorbutic diet, whilst a dose of 25 mg., which is capable of fully saturating the tissues of the animals during the pre-experimental period, should be unable to do so when the scorbutic diet did not contain such traces of the vitamin. This point requires further investigation.

The phosphatase content of liver, kidney and intestine in scurvy

As was seen in the preceding experiments the drop in the phosphatase concentration of the serum was almost synchronous with the first appearance of scorbutic lesions, whilst that of the bones did not fall to any significant extent until about 5 days later when the guinea pigs were also beginning to lose weight. Since the origin of the alkaline phosphatase of the serum in normal young guinea pigs is not reliably established, it was of interest to institute some exploratory experiments in order to ascertain the influence of the development of scurvy on the concentration of the enzyme in other tissues, particularly whether disappearance of phosphatase in any of these tissues at the onset of scurvy ran parallel to that of the serum phosphatase. The kidney, the liver and the large and small intestines were examined for this purpose, and the results are incorporated in Table 4. After 15 days on the scorbutic diet, when the phosphatase concentration of the serum had dropped to about a half its original value (cf. Fig. 1), no significant fall had occurred in the

concentration or total amount of the kidney or liver phosphatase. After 21 days, in the premortal phase, when the scorbutic lesions were acute and the fall in weight of the animals was very great, a fall in the concentration and in the total content of the enzyme ($t = 3.39$, $P < 0.01$; $t = 3.25$, $P < 0.01$) of the kidney only was observed. The large and small intestines were not analysed after 15 days, but these tissues did not show any fall in the phosphatase content even in the premortal phase.

Our results are therefore in agreement with those obtained by Russell, Rouse & Read (1944), who found in addition that scurvy did not bring about a fall of phosphatase in the spleen, lungs and adrenals. These results indicate, therefore, that the early drop in the phosphatase concentration of the serum during the onset of scurvy in growing guinea pigs maintained on a scorbutic diet is not reflected in these tissues, which are rich in phosphatase.

The influence of the administration of L-ascorbic acid to scorbutic guinea pigs on the phosphatase content of some of their tissues

In a preceding section results were given showing the influence of the development of scurvy on the phosphatase content of the serum, costochondral junctions and epiphyses of young guinea pigs (Fig. 1). In this section the immediate effect on these tissues of the administration of L-ascorbic acid to scorbutic guinea pigs is described. A dose of 25 mg. L-ascorbic

Table 4. *The phosphatase content of the liver, kidneys and intestine of guinea pigs on mixed and scorbutic diets*

		Kidneys					Liver				
				Phosphatase					Phosphatase		
Group	Diet	No. of animals		Wt. (g.)	Per g.	Whole tissue	No. of animals		Wt. (g.)	Per g.	Whole tissue
1	Mixed diet	10	Mean	2.58	2.33	5.96	6	Mean	14.9	0.31	4.53
			S.D.	—	0.75	1.76		S.D.	—	0.08	1.29
2	15 days scorbutic diet	6	Mean	2.64	2.05	5.35	6	Mean	13.5	0.28	3.70
			S.D.	—	0.54	1.47		S.D.	—	0.06	0.73
3	21 days scorbutic diet	8	Mean	2.76	1.27	3.51	8	Mean	11.6	0.34	3.84
			S.D.	—	0.40	1.35		S.D.	—	0.06	0.59
Comparison of groups 1 and 3			<i>t</i>		3.39*	3.25*		<i>t</i>		0.85	1.40
			<i>P</i>		<0.01	<0.01		<i>P</i>		0.4-0.5	0.1-0.2
		Large intestine					Small intestine				
				Phosphatase					Phosphatase		
Group	Diet	No. of animals		Wt. (g.)	Per g.	Whole tissue	No. of animals		Wt. (g.)	Per g.	Whole tissue
1	Mixed diet	4	Mean	8.9	1.57	13.7	4	Mean	8.8	9.07	80
			S.D.	—	0.73	5.62		S.D.	—	4.53	40
3	21 days scorbutic diet	4	Mean	9.4	1.91	17.9	4	Mean	8.4	11.2	98
			S.D.	—	0.36	3.28		S.D.	—	6.0	58
Comparison of groups			<i>t</i>		0.84	1.29		<i>t</i>		0.58	0.51
			<i>P</i>		0.4-0.5	0.2-0.3		<i>P</i>		0.5-0.6	0.6-0.7

* Denotes a significant difference.

acid daily was administered orally to animals which had been maintained for 18 and 21 days respectively on the scorbutic diet. The effect of the cure was observed in groups of animals after 2 and 4 days in each case. Fig. 6 gives a graphical representation of these results as well as the trends of the average weights derived from the daily weighing of the animals in each group. The important point to be noted is that there was a striking increase in the phosphatase content of all the tissues examined, and this cannot be attributed to the rise in weight of the animals since the growth was insufficient to produce such an effect. Consequently the increase must be due to the amelioration of the scorbutic condition of the animals.

Fig. 6. The effect of daily curative doses of 25 mg. L-ascorbic acid on the phosphatase content of the serum, costochondral junctions and epiphyses of young scorbutic guinea pigs. I, after 18 days subsistence on the scorbutic diet; II, after 21 days subsistence on the scorbutic diet. Trend of the average daily weights of the animals, ---; phosphatase activity of the serum, ○—○; costochondral junctions, □—□ and epiphyses, △—△. M.D., phosphatase values obtained with animals maintained on a mixed diet with cabbage *ad lib*. Six animals were used in each group.

The sharp increase in the enzyme content in all the tissues was already marked after 2 days, irrespective of whether the animals had been on the scorbutic diet for 18 days, when the syndrome of scurvy was fully developed, or for 21 days when the lesions in this premortal phase were very acute. The phosphatase contents of the bones, but not that of the serum, had after this short time of treatment reached values which were not significantly lower and were sometimes even significantly higher than

those of animals on a mixed diet with cabbage *ad lib*. (18-day group: serum, $t=2.91$, $P<0.01$, lower; costochondral junctions, $t=0.62$, $P=0.5-0.6$; epiphyses, $t=1.98$, $P=0.05-0.1$. 21-day group: serum, $t=5.46$, $P<0.01$, lower; costochondral junctions, $t=1.84$, $P=0.05-0.1$; epiphyses, $t=2.36$, $P=0.02-0.05$, higher). These figures also indicate the possibility that the maximum values for the epiphyses are reached after 2 days' treatment. The tissues of all the animals were found not to be fully saturated with L-ascorbic acid. Another point to be noted is that after 18 days on the basal diet the phosphatase content of the epiphyses, observed after 4 days' treatment with L-ascorbic acid, was significantly higher than that of animals on a mixed diet with cabbage *ad lib*. ($t=2.75$, $P<0.01$). Similarly, after 21 days on the basal diet and 4 days' administration of L-ascorbic acid this phenomenon was observed in the costochondral junctions ($t=2.66$, $P=0.01-0.02$). At the post-mortem examination it was found that although all the haemorrhages were mostly discoloured even after 2 days' treatment, vestiges of fresh haemorrhages were still present in almost all of the animals of this experiment.

In order to ascertain whether the increase of L-ascorbic acid in the tissues caused by the administration of the vitamin was responsible for the dramatic increase in the phosphatase content of the tissues the following experiment was performed. A group of six animals maintained for 18 days on a scorbutic diet were injected intraperitoneally with 25 mg. L-ascorbic acid and sacrificed 18 hr. later, and their tissues examined for phosphatase content. With the possible exception of two animals in this group no change in the haemorrhages was evident to the naked eye. The phosphatase figures obtained were as follows: serum, 5.5; costochondral junctions, 13.1; epiphyses, 13.2. When these figures are compared with the corresponding values of animals maintained for 18 days on a scorbutic diet (see Fig. 6) it is seen that they are not significantly higher (serum, $t=0.58$, $P=0.5-0.6$; costochondral junctions, $t=2.11$, $P=0.05-0.1$; epiphyses, $t=2.06$, $P=0.05-0.1$). Evidently the sharp increase in the phosphatase values observed above is not due to L-ascorbic acid *per se*, but to the therapeutic action of the vitamin.

Gould & Shwachman (1941) tried the effect of the administration of L-ascorbic acid for varying periods to nine scorbutic guinea pigs. No details were given of the time the animals subsisted on the scorbutic diet preceding the cure and the daily doses of L-ascorbic acid offered to the animals also varied with each individual guinea pig. They observed an increase in the phosphatase content in the sera and tibiae of all the animals and in the ribs of only three guinea pigs. Owing to the limited number of animals employed by them, to the lack of experimental

details given and to the different procedure adopted by them in the estimation of phosphatase we are unable to compare their results with ours.

DISCUSSION

An incontrovertible chain of direct and indirect evidence has been produced in this investigation showing that the fall in the phosphatase content in the serum, and in the zones of provisional calcification of the costochondral junctions and the tibiae observed during the development of scurvy in growing guinea pigs is caused by the scorbutic lesions, although in the later stages the fall in weight of the experimental animals may be a contributing agent. After 12 days on the scorbutic diet, very soon after macroscopic haemorrhages first appeared in some of the animals of the groups, but when all the animals were showing uninterrupted growth, the phosphatase of the serum fell by about 30 % of its original value. Furthermore, when growth was resumed after the restriction of the scorbutic diet for 9 days (Fig. 3) the phosphatase in the serum did not increase, as was the case in animals of similar growth receiving L-ascorbic acid (Fig. 4), but remained more or less constant for the succeeding 6 days, after which time the rapid fall was resumed. Evidently the fall in the serum phosphatase between the ninth and the fifteenth days was in this case balanced by the increase in the serum phosphatase due to the growth produced by the temporary excessive intake of food. The fall in the phosphatase content of the bones took place later than in the serum, between the eighteenth and twenty-first days on the scorbutic diet. This fall was not observed in scorbutic adult guinea pigs, probably because of the low osteoblastic activity of the bones; the serum of such animals, however, showed a definite diminution of the phosphatase concentration. The close relationship between the development of scurvy and the fall of phosphatase in the tissues is brought out in a striking fashion by the experiment in which the onset of the disease was delayed by a few days owing to the modification introduced by restricting growth during the first 9 days (Fig. 3). In this case the drop in the phosphatase content of the tissues was accordingly delayed.

Although the decrease in the phosphatase content of the bones always took place in the animals maintained on the scorbutic diet at a time when they were beginning to lose weight, it is nevertheless evident that it was caused essentially by the scorbutic condition and not by the loss in weight. This is seen from the fact that a similar fall in weight did not cause a drop in phosphatase content in the tissues of the animals which received L-ascorbic acid (Fig. 4). Also when L-ascorbic acid was administered to guinea pigs after 18 and 21 days on a scorbutic

diet, the bone phosphatase rose very sharply to normal or even higher values after 2 days dosing only, while the weight of the animals hardly responded to the administration of L-ascorbic acid during this time. Further evidence of the differential behaviour of the phosphatase in these tissues caused by the fall in weight and by scurvy in growing guinea pigs is illustrated by the following facts. In the first place in scurvy the serum phosphatase fell to a much greater extent than did the phosphatase in the bones. Thus after 21 days on a scorbutic diet the serum phosphatase had fallen by as much as 84 % from the mixed diet value, whereas the costochondral junction phosphatase had fallen 52 % and that of the epiphyses 49 %. A similar relationship was maintained during the early stages of treatment with L-ascorbic acid (Figs. 1 and 6). On the other hand, after a fall in weight in the absence of scurvy, this relationship was not maintained since all the three tissues were affected to about the same extent. Thus after 9 days on a restricted scorbutic diet with 25 mg. L-ascorbic acid daily the results were as follows: fall in the phosphatase content of the serum 58 %, of the costochondral junctions 53 % and of the epiphyses 50 % (Fig. 2). In other words, the phosphatase of the serum fell relatively to that of the bones to a much smaller extent than in the case of the scorbutic animals.

The disappearance of the serum phosphatase during the development of scurvy requires further consideration. It would not be unreasonable to assume that as soon as early pathological changes occur the serum phosphatase begins to be transported under stress to the tissues affected. Considering that the diminution in the serum phosphatase coincides closely with the macroscopic appearance of haemorrhages it is also probable that the tissues of the vascular system are the likely objective. If this assumption be correct and if the serum acts solely as a medium for transport of alkaline phosphatases originating in other tissues, it is surprising to find that the original concentration of the serum is not maintained by further supplies from the tissues in which the serum enzyme is supposed to originate and that a diminution in the phosphatase content of these tissues should not ensue. We have found that no such diminution took place, unless in quantities too small to be detected by our methods, in the bones, the liver, the intestine and the kidneys of the animals after subsisting for 15 days on the basal diet. These observations, therefore, if one excludes the improbable contingency that owing to damage these tissues are prevented from supplying phosphatase to the blood stream, militate against the conception that under normal conditions the greater part of the serum phosphatase originates in one or several of these tissues and that the serum just mediates the transport of

the enzyme to other tissues. The present evidence does not even exclude the possibility that the serum phosphatase under normal conditions may be mainly of vascular origin and that it is removed from the circulation under stress due to certain incipient pathological changes.

That L-ascorbic acid *per se* is not the regulating agent of the phosphatase content of the tissues is seen from the fact that 18 hr. after the intraperitoneal injection of 25 mg. which completely saturates the tissues, no rise of the phosphatase content of the tissues of scorbutic animals was observed. Only after 2 days' oral consumption, when the therapeutic action of the L-ascorbic acid was in progress, was a striking rise in the content of the three tissues observed. The fact that guinea pigs which received a daily dose of 2 mg., i.e. animals which were protected from scorbutic lesions, but whose tissues contained little L-ascorbic acid, showed normal phosphatase content in the serum, costochondral junctions and the epiphyses, also supports this view.

The speed with which the phosphatase content of the tissues rises after the administration of L-ascorbic acid to scorbutic guinea pigs is very striking. The values obtained with most of the bones, after dosage for 4 days, were higher than those obtained in guinea pigs maintained on a mixed diet with cabbage *ad lib*. It would be interesting to ascertain whether these raised values are capable of returning to normal on continuing the administration of L-ascorbic acid. It is also to be noted that the serum phosphatase, unlike that of the bones, did not reach normality after 2 days' dosing. The serum phosphatase therefore is the first of the phosphatases of the three tissues to fall during the development of scurvy and the last to reach its normal level after cure, which suggests again that it possibly fulfils some protective function and that it differs in this respect from the bone phosphatases. In the latter case the fall may be due to a disturbance in the function of the osteoblasts caused by local scorbutic lesions. It is therefore not surprising that in scorbutic adult guinea pigs the serum phosphatase is diminished as it is in the young guinea pigs, although to a less degree. The scorbutic lesions, however, could not have been expected to affect the phosphatase content produced by the quiescent osteoblasts of adult animals to a noticeable extent. Although it cannot be excluded that the fall in serum phosphatase in scurvy may also be due to damage in the tissue, the evidence so far available rather points to the probability that it is associated with a protective function. In this connexion it is of importance to note that the fall of phosphatase in these tissues incurred during loss of weight by young guinea pigs uncomplicated by scurvy does not indicate such differentiation between the serum and bone phosphatases.

It is assumed that all the changes in the phosphatases were due to the enzyme proper and not to any activator or activators. The constant increase in the relative activity brought about by the addition of magnesium in all the experiments of this investigation strengthens this assumption.

SUMMARY

1. The serum phosphatase of young guinea pigs began to fall after the animals had subsisted on a scorbutic diet for 12 days, at a time when their growth was uninterrupted. The phosphatase content of the costochondral junctions and of the epiphysis of the tibia with the adjoining cartilage did not show any fall before 15–18 days. This latter fall was probably brought about mainly by scorbutic lesions and not by loss in weight.

2. The administration of a daily dose of 25 mg. L-ascorbic acid to scorbutic guinea pigs maintained for 18 or 21 days on the deficient diet raised the phosphatase content of the three tissues very rapidly. Two days' treatment with L-ascorbic acid increased the phosphatase content of the bones, but not of the serum, to that of normal animals. Further 2 days' treatment brought the phosphatase content of the serum up to normal values and those of most of the bones to values above those obtained in guinea pigs subsisting on a mixed diet with cabbage *ad lib*.

3. Maintenance of guinea pigs on a scorbutic diet supplemented with 2 mg. of L-ascorbic acid daily, a dose not sufficient to saturate the tissues, but just adequate to prevent the development of scorbutic lesions, caused no diminution in the tissue phosphatase content.

4. Guinea pigs receiving a scorbutic diet and daily doses as high as 25 mg. L-ascorbic acid showed, 10 days after the withdrawal of L-ascorbic acid, phosphatase values in the three tissues lower than those observed in animals which received cabbage *ad lib*. in the preliminary period. Yet the course of the development of scurvy in both cases was identical. The lower values thus obtained could be brought up to normal by the administration of a daily dose of only 0.05 mg. L-ascorbic acid during the 10 days' existence on the scorbutic diet.

5. The phosphatase content of the tissues was found not to be raised in scorbutic guinea pigs 18 hr. after the intraperitoneal injection of 25 mg. L-ascorbic acid which causes saturation of the tissues. It is therefore not the direct regulating agent of the phosphatase content of the tissues.

6. The existence of guinea pigs on a scorbutic diet for 15 days had no influence on the phosphatase content of the liver, intestine and kidneys. The latter showed a fall in the premortal phase.

7. The fall in the phosphatase concentration of the serum of guinea pigs caused by a fall in weight of

the animals and not by scurvy was very much less marked than that observed in scorbutic guinea pigs.

8. It is assumed that the fall of the phosphatase in the zones of provisional calcification of scorbutic guinea pigs may be due to a disturbance in the function of the osteoblasts caused by scorbutic lesions. The evidence so far obtained, however, makes it probable that the early diminution in the serum phosphatase is not due to a disturbance in the pro-

duction of the enzyme, as in the case of the bones, but rather to its removal from the circulation to fulfil some protective function. The evidence also does not exclude the possibility that the serum phosphatase of healthy animals is of vascular origin.

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Chemical Studies of Peripheral Nerve During Wallerian Degeneration

2. LIPIDS AFTER NERVE CRUSH (AXONOTOMESIS)

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In a previous paper the changes that occur in the lipids of a peripheral nerve during Wallerian degeneration were described (Johnson, McNabb & Rossiter, 1949b). The sciatic nerve was sectioned in a number of cats and the peripheral segment was allowed to degenerate. Special precautions were taken to minimize the possibility of re-innervation. In the experiments now to be reported the nerve was gently crushed, a procedure that causes the distal segment to undergo a series of changes similar to those of Wallerian degeneration. After a short latent period the degenerating nerve is then re-innervated by axon tips from the intact central stump. This type of lesion has been called axonotmesis by Seddon (1943) and must be distinguished from neurotmesis, the type of lesion previously investigated.

The changes in the nerve are essentially those of Wallerian degeneration, upon which are superimposed those of regeneration. Previous studies in this field have been for the most part either histological or physiological. Excellent reviews are those

of Howell & Huber (1892), Kirk & Lewis (1917), Ramon y Cajal (1928), Spielmeyer (1929), Nageotte (1932), Weiss (1944) and Young (1942, 1945, 1948). In few instances have the techniques of chemistry been employed. The most notable exceptions are the studies of Mott & Halliburton (1901), who measured the phosphorus content of regenerating cat nerves; and Abercrombie & Johnson (1947), who reported changes in the distribution of nitrogen, including collagen nitrogen, in regenerating rabbit nerves.

METHODS

The right sciatic nerve of thirty-eight adult cats was gently crushed at the level of the greater trochanter of the femur with a crushing clamp. This procedure interrupts the axons and the myelin sheaths, leaving the connective-tissue sheaths intact. The operation was performed under nembutal anaesthesia with full aseptic precautions. No attempt was made to control the age, sex, or weight of the animals. After periods of time varying from 16 to 144 days the animals were killed and the segment of the nerve distal to the site of the crush was removed. At the same time, a similar length