CORTISONE - ASCORBIC ACID INTERACTION AND THE PATHOGENESIS OF AMYLOIDOSIS*†

MECHANISM OF ACTION OF CORTISONE ON MESENCHYMAL TISSUE

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The experiments to be reported here show that under certain conditions a few injections of cortisone into animals result in the prompt appearance of amyloid in the spleen or other organs; they indicate that the mechanism whereby the hormone of the adrenal cortex maintains the structure and function of mesenchymal tissue in general is an *indirect* one, mediated by ascorbic acid. Well-defined structural and functional phases in the natural diseases and in the experimental disorder of the mesenchyma—which may in some way be associated with a disturbance of some enzyme system (ribonuclease, hyaluronidase)—seem to be controlled by an *interaction* of a hormone of the adrenal cortex of cortisone type with ascorbic acid.

These findings offer an approach to the elucidation of the following points:

- (i) *The pathogenesis of amyloidosis*, including the origin of amyloid in rheumatoid arthritis,
- (ii) *Certain apparently antagonistic effects of cortisone and ascorbic acid on mesenchymal tissue*,
- (iii) *The mechanism of action of cortisone and ascorbic acid on mesenchymal tissue in general*.

The causation and mechanism of the formation of amyloid is still considered to be very much of a mystery, and though many different theories have been put forward in rapid succession, it has been impossible to establish a common denominator for the formation of amyloid in the series of varying conditions in which it has been observed in human as well as in experimental studies.

An old theory was that amyloid was formed when chondroitin-sulphuric acid, released from the breaking down of cartilage or elastic tissue, was combined with protein. Loeschcke (1927) looked upon amyloid as an insoluble precipitate of antigen and antibody. It has been generally believed that amyloid is an infiltration rather than a degeneration.

The protein content of amyloid has for a long time suggested that some abnormality of the protein metabolism is involved, and conditions in which secondary amyloidosis may develop are often associated with hyperglobulinaemia. The

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morphogenetic relations of hyalinosis, amyloidosis, and paramyloidosis in mesenchymal disease associated with hyperglobulinaemia and accumulations of plasma cells in the active phase have recently been studied in detail (Teilum, 1948a, b; 1949).

The association of amyloidosis and rheumatoid arthritis is rare (Hench and others, 1948). Unger and others (1948) found amyloidosis in four out of 58 cases examined *post mortem*, and in the Congo red test in six out of 56 patients. In a series of *post-mortem* examinations comprising about 100 cases of rheumatic and pararheumatic disease we have found an astonishingly high incidence of amyloidosis in rheumatoid arthritis (Teilum and Lindahl, 1952) in the Laboratory for Rheumatic Research. Amyloid deposits, most frequently present in kidney, spleen, and adrenal glands, could be demonstrated in twenty out of 32 cases (Table I). The changes were pronounced in eleven cases, but milder degrees were revealed by means of methyl violet stain for amyloid in the other nine cases. The occurrence of milder or severer degrees of amyloid deposition was thus found to be a highly characteristic lesion in rheumatoid arthritis, and presumably one of the most frequent causes of amyloid formation in general.

TABLE I
INCIDENCE OF AMYLOID LESIONS IN 32 CASES OF RHEUMATOID ARTHRITIS
(after Teilum and Lindahl, 1952)

Grade	No.	Site	No.
Advanced	8	Kidneys	15 (inc. 9 adv. or mod.)
Moderate	3	Spleen	16
Mild	9	Adrenals	5
Total cases	20		

Experimentally, amyloidosis has been produced by various means, including repeated parenteral administration of protein foreign to the species, repeated injections of antigen, bacteria, casein, sodium caseinate, pentose, nucleotides, human serum, sulphur, etc., and it has also been found in tumour-bearing mice. Recently Pirani and others (1949) demonstrated amyloid in guinea-pigs fed on scorbutogenic diets for 8 weeks or longer. Six out of seven animals of this group showed distinct amyloidosis, and in those showing amyloid deposition the spleen was severely involved, the liver moderately, and the adrenal cortex only minimally in few cases. According to these authors, their observations do not warrant any positive conclusion as to the possible role of ascorbic acid or inanition in the pathogenesis of amyloid deposition, but they point out that amyloidosis has not been produced previously in animals by means of a deficient diet.

Variability in staining reactions suggests that amyloid is not a uniform chemical substance, but a series of closely-related protein compounds (Hass and others, 1943), the composition of which may vary from one case to another and in different areas within the same case. Letterer (1949), who studied the electrophoretic pattern of serum protein in amyloid mice, believes that the protein constituent of amyloid is not of a specific chemical nature, but a part of plasma protein, and in many cases the result of antigen-antibody reactions. Two processes appeared to be of importance: the disturbance of the colloidal stability of the plasma, and the formation of new plasma proteins of α , β , and γ types (Letterer, 1949).

Much uncertainty has surrounded the chemical nature of the amyloid substance,

but at the present time it is generally considered to be a *glycoprotein* in which a mucopolysaccharide (chondroitin sulphuric acid (Ehrström, 1939) or mucoitin monosulphuric acid (Meyer, 1947)) has been attached to a globulin.

Hass (1942) identified two slightly different protein fractions and a sulphate-bearing polysaccharide in secondary amyloidosis, and showed that from 1 to 2 per cent. of the amyloid molecule is of carbohydrate nature. It was concluded that amyloid has a matrix of protein which becomes complex by combination with various other substances of the body fluids.

In some cases Johansson and Wahlgren (1938) has found a metachromatic staining of amyloid with toluidine blue.

Experiments

Pyroninophilic Mesenchymal Cells and Origin of Hyaline and Amyloid.—A further elaboration of my previous studies on hyalinosis and amyloidosis in the glomeruli of the kidney, the spleen, and other organs in mesenchymal disorders also associated with hyperglobulinaemia and accumulations of plasma cells (Teilum, 1948-49), shows that cytoplasmatic pyroninophilia of reticulum cells and other mesenchymal derivative cells represents a common alteration in the early phase of mesenchymal disorder, which may result in the formation of hyaline or amyloid.

The findings are illustrated by the following examples of mesenchymal disease and by experiments in animals.

Sarcoidosis (Teilum, 1948a, 1949, 1951) pyroninophilic mesenchymal cells—in addition to an accumulation of typical plasma cells—were found scattered or accumulated in lungs, spleen, lymph nodes, and other organs, and the pyroninophilic substance showed all transitions to prehyaline and hyaline masses. The so-called stratified intracellular bodies in sarcoidosis were shown to be pyroninophilic in their early stage and were considered to be a biochemical product of mesenchymal cells related to the pronounced development of hyalinosis (Teilum, 1949). Hyaline or prehyaline glomerular lesions with the same pathogenesis and explaining the renal disease in sarcoidosis were later described (Teilum, 1951).

Pyroninophilic cells and giant cells with pyroninophilic inclusions in the lung were a common finding in generalized sarcoidosis. Sections from the same blocks were incubated with purified ribonuclease (10 mg.-100 ml.) at pH 6·7, or with buffer solution alone for 1 hour at 37° C. After treatment the sections were stained with pyronine-methyl-green and the incubated sections were compared with the untreated sections. After incubation the pyroninophilia of the cytoplasm of the different mesenchymal cell types was abolished.

Glomerulonephritis. Studies of sections from acute and subacute cases of glomerulonephritis and renal changes in paramyloid syndrome with hyperglobulinaemia (Teilum, 1948b) also revealed, in addition to plasma cells, pyroninophilic cells of the glomerular tufts (epithelial and endothelial), interstitial mesenchyma, adventitia of vessels, and vascular endothelium. All stages of transition from such cytoplasmatic changes to prehyaline, hyaline, or paramyloid substance were observed in different cases.

It was evident that formation of hyaline, amyloid, and related substances was always anticipated—in glomeruli, interstitium, vascular walls—by a *pyroninophilic precursor stage* of mesenchymal cells, representing a typical alteration in the *active phase*, which is also characterized by the accumulation of plasma cells in the spleen and other organs, and by a liberation of γ -globulin to the blood.

Teilum and others (1950) recently described a pronounced inhibitory effect of cortisone on accumulation of plasma cells in the spleen, as well as on pyroninophilic cells in the glomeruli of the kidney in experimental acute glomerulonephritis in rabbits hyperimmunized

for several months (1951). In the animals treated with cortisone, there was a marked rise in the α -globulin fraction and a less marked fall in γ -globulin. In the other series not so treated, the γ -globulin was elevated in those animals who had acute nephritis before treatment with cortisone was commenced, whereas the cases with a marked nephrotic syndrome showed an increase of α - and β -globulin fractions and decreased total protein (Teilum and others, 1951).

In the animals not treated with cortisone a more protracted transition can be seen

FIG. 1.—Transition from swollen pyroninophilic glomerular cells to a non-pyroninophilic homogeneous (pre-hyaline) substance. Hyperimmunized rabbit. Pyronin-methylgreen. $\times 950$.

FIG. 2.—Pyroninophilic cells in epithelium of visceral and parietal capsule layer of the glomerulus. Note larger lump of nuclei surrounded by a pyroninophilic cytoplasmic mass in centre. Hyperimmunized rabbit. Pyronin-methylgreen. $\times 950$.

from swollen glomerular cells with blurred, highly pyroninophilic cytoplasm to a non-pyroninophilic pale homogeneous prehyaline or pre-amyloid substance (Fig. 1), whereas the administration of cortisone resulted in the prompt appearance of the last-mentioned phase. Fig. 2 shows pyroninophilic cells in the epithelium of the visceral and parietal capsular layer with swollen cytoplasm, and in glomerulus a larger lump of pyroninophilic cells with many nuclei, surrounded by an ill-defined blurred pyroninophilic cytoplasmic mass. The pronounced pyroninophilia of the glomeruli rich in cells in experimental acute glomerulonephritis was also abolished by treatment with ribonuclease as described above (Figs 3 and 4).

FIGS 3 and 4.—Sections from same block of experimental acute glomerulonephritis in rabbit. Pyroninophilia of cytoplasm of glomerular cells abolished after incubation with ribonuclease. (Cf. Fig. 4 with the untreated section, Fig. 3.) Pyronin-methylgreen. $\times 320$.

Whilst the active (pyroninophilic) phase of mesenchymal disorder is thus linked with an increased production of cytoplasmatic protein (ribose nucleic acid) by reticulum cells and other mesenchymal derivative cells, which in natural disease—under the influence of controlling factors—will show regression in its further course with or without formation of hyaline (*hyalinosis*) representing an inactive phase of healing, a perverted phase in mesenchymal protein synthesis may, under certain circumstances, take place and result in the formation of amyloid (*amyloidosis*).

Effects of Cortisone in Producing Amyloidosis

Letterer (1926) and Bohle and others (1950) produced experimental amyloidosis in mice by means of repeated injections of sodium caseinate or of nucleic acid solution. The incidence of amyloidosis in such experiments seems, however, to be varying, and Hass and others (1943) failed to produce amyloidosis in mice which were given injections of a solution of sterile sodium caseinate.

Material and Methods.—In these experiments, female mice of the same C₃H-strain weighing from 20 to 25 g. were used. During the experiment and for the preceding 8 days, they were only given oatmeal and water. They then received 0.5 ml. of a 2 per cent. casein solution in 0.25 per cent. NaOH in daily hypodermic injections into the back. In the course of 3 weeks, three series of five injections were given with intervals of 2 days between each series, i.e. a total of fifteen injections in 21 days; this was followed by up to ten injections in the course of another 4 or 5 weeks. The first experiments comprised 160 mice. Some of these had, *in addition*, been given 0.3 mg. cortisone in hypodermic injections simultaneously with the casein injections for the purpose of examining the influence of this hormone on the development of experimental amyloidosis at various times in the course of treatment, the changes of the spleen were controlled by means of biopsy, made under ether anaesthesia through a laparotomy with ligation of the distal pole of the spleen. The animals were weighed three times a week. Blocks were taken from the spleen, kidneys, adrenals, and liver, being fixed in 10 per cent. neutral formalin and then embedded in paraffin. Sections were stained with hematoxylin-eosin, by Van Gieson's method, with toluidine blue, and by the Hotchkiss periodic acid routine, as well as by the Unna-Pappenheim pyronin-methyl-green method for pyroninophilia and the Congo red stain and methyl-violet stain (Eden) for amyloid.

Results.—After casein injections had been given for 3 weeks, biopsy of the spleen of seven subjects revealed no signs of amyloidosis. After 5 weeks, four other subjects treated with casein injections only showed no signs of amyloidosis of the spleen, whereas four of the cortisone-treated animals killed at this time displayed it to a marked degree.

Similarly, five casein-treated mice showed no amyloidosis 6 weeks after beginning the treatment, whereas five casein-cortisone-treated mice displayed marked amyloidosis of the spleen.

As these findings seemed to indicate that cortisone promoted the production of amyloid, eight casein-cortisone-treated mice and another eight mice treated with casein solution only were killed 8 weeks after beginning the treatment. In the latter group none showed signs of amyloidosis of the spleen, but only some reticulum cell proliferation, whereas all those in the former group showed marked amyloidosis of the spleen, and some showed rather less pronounced changes in liver and kidneys.

In another experiment, eight mice treated in advance with casein only for 6 weeks

FIG. 5.—Biopsy of spleen in mouse treated with casein injections for 6 weeks and showing no signs of amyloidosis. Haematoxylin and eosin. $\times 160$.

FIG. 6.—Marked amyloidosis in spleen of same animal as Fig. 5 after further administration of 0.3 mg. cortisone daily for 4 days. Haematoxylin and eosin. $\times 160$.

displayed no signs of amyloidosis of the spleen at biopsy; four of these animals were then given 0.3 mg. cortisone daily for 4 days; they were then killed, and a very pronounced amyloidosis of the spleen produced by the few days' administration of cortisone (Figs 5 and 6) was found histologically at autopsy, whereas there were no amyloid deposits in the four animals not treated with cortisone.

Only two out of 22 casein-treated mice which had not been given cortisone

displayed pronounced amyloidosis in the spleen or in other organs 8 weeks after the casein injections were begun.

Daily injections of 0.3 mg. cortisone for 6 days did not cause amyloidosis in eight subjects not treated with casein in advance. Amyloidosis did not develop after administration of cortisone in animals treated with casein only 2 weeks in advance, but appeared in seven out of eight animals treated for one month.

A similar but essentially weaker and less constant amyloid-producing effect was found after the administration of ACTH (Acton) 0.5 mg. administered in two daily doses for 5 days to five mice.

The time of onset of the amyloid-producing effect of cortisone in relation to the beginning of the casein treatment shows some variations in the various series of experiments, but it is evident that the administration of cortisone resulted in the prompt appearance of amyloid in mice previously treated with injections of sodium caseinate for several weeks, though the casein had been insufficient to produce amyloidosis by itself.

In connection with the injection treatment, some of the subjects developed ulcerations and necroses around the site of injection, and in a few of the experiments there was quite a high mortality among the animals, which often showed a very poor resistance.

After a few weeks' casein injections the spleen showed accumulations of pyroninophilic reticulum cells, including numerous plasma cells and pyroninophilic giant cells with irregular arrangement of the nuclei. The cortisone treatment caused a depression of the pyroninophilia with deposition of homogeneous amyloid masses, beginning in the perifollicular zone, other generations of cells gradually passing through the same phases until the greater part of the pulp was involved. Though during the early stages the deposits form more or less well-defined globular precipitates corresponding to the cellular origin (Fig. 6), the continued administration of cortisone caused a deposition of completely confluent masses without any remnants of cellular structure (Fig. 8). The deposits gave a positive reaction with Congo red, but only a few cases reacted with Eden's methyl violet stain, and none with toluidin blue or Hotchkiss periodic acid stain. After prolonged treatment with casein or casein followed by cortisone, there also appeared more or less pronounced deposits in the liver, the glomeruli of the kidneys, and the adrenal glands, indicating a weaker and later pyroninophilia of the mesenchymal derivative cells in these organs than that in the spleen.

The cortisone treatment caused a reduction of the adrenal glands, which had become hypertrophic after the preceding casein treatment.

Effect of Cortisone in Producing Amyloidosis in Rabbits

In a previous paper (Teilum and others, 1950), the findings in hyperimmunized rabbits after treatment with cortisone were described. Besides a marked regression of massive accumulations of plasma cells in the spleen in some cases a homogeneous substance appeared in the perifollicular zone in the spleen. Even if it failed to give characteristic staining reactions for amyloid, which is often the case in experimental amyloidosis, the localization and appearance were quite characteristic, and these cases certainly presented an amyloid alteration. Where this

change in the spleen had been ascertained by biopsy before administration of cortisone a marked increase of the depositions occurred afterwards.

With this may be compared the development of amyloid nephrosis in cortisone-treated rabbits with nephrotic syndrome (Teilum and others, 1951).

In mice treated in advance with repeated injections of casein, and in rabbits hyperimmunized for many months with killed Pfeiffer bacillus culture, cortisone has essentially the same effect: inhibition of pyroninophilic cells (including plasma cells), appearance of reticulosis where the cells display a pale, homogeneous, non-pyroninophilic substance, and—in certain cases—a prompt appearance of amyloid beginning in the perifollicular zone of the spleen.

Morphological Phases in Formation of Amyloid

The morphological studies at various stages of the amyloid synthesis in the spleen and of the influence of cortisone and ascorbic acid on this synthesis show that pronounced pyroninophilia of mesenchymal derivative cells in the spleen and other organs is fundamental to the pathogenesis of amyloidosis, depending in degree on the preceding pyroninophilia. As long as the amyloidosis developed after an antigen (casein injections or hyperimmunization) has not reached its maximum, pyroninophilic cells occur simultaneously around the amyloid masses already formed. In cases which do not later display any tendency to amyloid deposition, slight pyroninophilia is found in biopsy material from the spleen. The pyroninophilic reaction is usually most pronounced in the reticulum cells of the spleen, but may also be seen in other mesenchymal derivative cells in the liver, the glomeruli of the kidneys, and the interstitial tissue, corresponding, that is, to all the usual localizations of amyloid or hyaline.

As a series of examinations have shown it to be highly probable that the plasma cells are capable of producing antibodies (Bing and Plum, 1937; Bjørneboe and Gormsen, 1943; Fagraeus, 1948; and Ehrich and others, 1949), and the pyroninophilic mesenchymal cells occur in parallel with the accumulations of pyroninophilic plasma cells in the reticulo-endothelial system, there seems to be a close pathogenetic relation between antigenic influences and the development of hyaline or amyloid (Teilum, 1948a, b).

The cytological findings in the spleen conform with the results of examinations of the electrophoretic pattern made by Letterer (1949) and Bohle and others (1950) in mice during the development of experimental amyloidosis. Letterer thus found a higher γ -globulin increase in amyloidosis-affected mice than in non-affected mice after fifteen injections of nucleic acid. Bohle and others found that the γ -globulin values in the serum rise sharply after twelve to twenty injections, whereas after thirty injections they are lower than the normal values. Compared with the subjects that did not develop amyloidosis, the mice affected after twelve to twenty injections had unquestionably elevated γ -globulin values, whereas those who developed it after thirty injections had lower γ -globulin values than those who were unaffected.

Our biopsies of the spleen show that widespread amyloidosis may develop in mice a few days after the administration of cortisone.

**Maintaining Effect of Ascorbic Acid on Pyroninophilic Cells
in the Spleen in Hyperimmunized Rabbits**

The interaction between ascorbic acid and cortisone and the occurrence of pyroninophilic cells in the spleen in hyperimmunized rabbits was examined in the following experiments. Eight rabbits, immunized with killed Pfeiffer bacillus culture for 6 to 12 months as previously described, were daily given varying doses of cortisone and ascorbic acid subcutaneously for 7 days, a few being treated with only one substance. Biopsy of the spleen was made on March 12, before treatment, and being treated for 7 days they were all killed on March 20. All the biopsies showed a moderate accumulation of plasma cells with incipient regression. Dosages of 5, 10, 15, and 20 mg. cortisone daily caused an almost complete regression of the plasma cells in the spleen in each case, in spite of the simultaneous administration of 333 mg. ascorbic acid daily by hypodermic injection, but whereas the control, which received neither ascorbic acid nor cortisone, also displayed some regression of the pyroninophilia 8 days after the biopsy, the rabbit which had received ascorbic acid without cortisone, presented a violent increase of pyroninophilic cells, which formed broad, proliferating zones perifollicularly and in the pulp (Fig. 7). This effect was later confirmed in a larger group of animals (Teilum and others, 1952).

Also, in experiments with casein-treated mice, which showed only a weak pyroninophilia at biopsy of the spleen, the pyroninophilia increased after 0.1 mg. ascorbic acid daily for 5 days, but this change did not appear in controls not treated with ascorbic acid.

FIG. 7.—Re-appearance of plasma cells and pyroninophilic cells in spleen after administration of ascorbic acid. Hyperimmunized rabbit. Pyronin-methylgreen. $\times 950$.

The action of ascorbic acid in causing amyloidosis in the spleen in casein-treated mice was shown in the following experiment:

Ten mice treated with injections of 0.5 ml. sodium caseinate for 5 days weekly from September 29 to November 6, showed very slight amyloid deposits in the spleen or none at all, whereas ten mice receiving 0.2 mg. ascorbic acid daily in addition showed a moderate amyloid deposition in the perifollicular zone.

This indicates that ascorbic acid activates the formation of pyroninophilic substance in mesenchymal cells and is thus of decisive importance in the production of globulins (and antibodies).

Casein- and cortisone-treated mice with a diffuse amyloidosis in the spleen at biopsy (Fig. 8), after a period of 4 weeks without any injections, showed a pronounced tendency for the amyloid changes to regress, so that they became more localized, with homogeneous depositions and masses characteristic of the early stages. After treatment with daily injections of 0.2 mg. ascorbic acid daily, the regression during this 4-week period was very pronounced (Fig. 9, overleaf).

Comment.—These results indicate that amyloidosis represents a dysfunctional phase in the activity of reticulo-endothelial and other mesenchymal cells, which display essentially the same alterations. An active *pyroninophilic* phase may be distinguished, corresponding to the active phase in such diseases of the mesenchymal tissue as rheumatic and pararheumatic conditions, sarcoidosis, and acute glomerulonephritis. This phase is characterized by:

- (i) the occurrence of pyroninophilic mesenchymal cells, which we have been able to demonstrate in various organs, such as the spleen, kidneys, and liver,

FIG. 8.—Biopsy of spleen in mouse treated with casein injections and further injections of cortisone showing diffuse amyloidosis (cf. Fig. 9, overleaf.) Haematoxylin and eosin. $\times 160$.

- (ii) the accumulation of plasma cells in the reticulo-endothelial system,
- (iii) elevated serum γ -globulin values with increased production of antibodies,
- (iv) the occurrence, in many cases, of metachromatic extracellular material.

Experimentally, all these morphological reactions have been accentuated after the administration of ascorbic acid. Further, it appears that all the changes characteristic of this phase are inhibited by the continued administration of cortisone in large doses. In certain cases this may produce hyalinosis, as, for instance, in the reticulum of the spleen (Teilum and others, 1950), and in cases of prolonged stress, for instance, in repeated stimulation of the immune mechanism, the cortisone treatment will result in the development of amyloidosis. This, then, may be distinguished as a negative phase, and is indicative of a perverted function of the mesenchymal cell with regard to the protein synthesis. Pirani and others (1949) succeeded in producing experimental amyloidosis in guinea-pigs fed on scorbutogenic diets for 8 weeks or longer. In addition to hyperplasia of the adrenal cortex, which must be considered an alarm reaction, Teilum and others (1952) found pyroninophilic cells in the spleen, which later underwent a transition to a pale, non-pyroninophilic, pre-amyloid reticulosis accompanied by changes in the electrophoretic pattern, showing the same morphological phase development as is seen in experimental amyloidosis caused by repeated stimulation of the immune mechanism and associated with similar changes in the adrenal cortex and serum globulins.

In amyloidosis produced in widely different ways the common denominator is thus seen to be a change in the protein synthesis of mesenchymal derivative

FIG. 9.—Regressive changes of amyloid in the spleen of same animal as Fig. 8 after further administration of ascorbic acid for 4 weeks. Haematoxylin and eosin. $\times 160$.

cells controlled by an interaction between ascorbic acid and a hormone of the adrenal cortex similar to cortisone.

Special attention has been given to the possible role of ascorbic acid in adaptation and the development of the General Adaptation Syndrome (Selye, 1950). The importance of ascorbic acid in connection with the pituitary-adrenal-cortex mechanism is obvious from the high concentration in the adrenal cortex and the rapid fall of ascorbic acid in response to pituitary hormone (Pincus, 1947). Dugal and Thérien (1949) found that, when large doses of ascorbic acid were given to guinea-pigs, the enlargement of the adrenals on exposure to cold was prevented, although the animals were more resistant than the untreated controls. According to this theory, the proliferation of the cortex has been claimed not as a direct effect of corticotropin, but as a local response to the exhaustion of sources of energy due to corticotropin stimulation of hormone synthesis and release.

Antagonistic Effects of Cortisone and Ascorbic Acid on the Mesenchymal Tissue

Since the appearance of cortisone it has been possible to ascertain its effects on the reactions of the mesenchymal tissue. On closer consideration it appears that these effects are actually identical with those previously found in experimental ascorbic acid deficiency. In addition to the antagonistic effects of ascorbic acid and cortisone on experimental amyloidosis and pyroninophilia (ribonucleic acid synthesis) of mesenchymal cells here described, the following points may be mentioned (Table II). A reduced capacity for wound healing and a delayed development of granulation tissue after the administration of corticotropin or cortisone have been observed in numerous cases in animal and man. This inhibition of all connective tissue elements conforms with the histological studies of Wolbach (1933) on the influence of vitamin C deficiency on wound healing in guinea-pigs.

TABLE II
ANTAGONISTIC EFFECTS OF CORTISONE AND ASCORBIC ACID ON MESENCHYMAL TISSUE

Effect on Mesenchymal Tissue	Cortisone	Ascorbic Acid
Experimental Amyloidosis	+	-
Plasma Cells	-	+
Pyroninophilic Mesenchymal Cells (Ribonucleic acid synthesis)	-	+
Development of Granulation Tissue ..	-	+
Wound Healing	-	+
Production of Acid Mucopolysaccharides ..	-	+

Furthermore, in the course of ACTH or cortisone treatment of patients with rheumatoid arthritis, a decrease of the metachromatic elementary substance in the connective tissue of the skin has been demonstrated (Asboe-Hansen, 1950); conversely, Penney and Balfour (1949) found that in guinea-pigs on a diet without vitamin C ascorbic acid produces a rapid increase of mucopolysaccharides in experimental wound healing.

All these strikingly antagonistic effects on the mesenchymal tissue of cortisone and ascorbic acid are indicative of an interaction which is normally present between

these two substances, which together maintain the normal function and structure of the mesenchymal tissue. A few clinical observations may presumably be explained by such an interaction. For example, daily doses of 4 to 6 g. ascorbic acid without simultaneous administration of steroid hormone had a surprisingly favourable effect in certain cases of polyarthritis (Berg, 1950; Massell and others, 1950).

The examinations by Irons and others (1951) of ascorbic acid metabolism during ACTH and cortisone treatment of mesenchymal diseases also seem to indicate that the utilization of ascorbic acid is increased.

Fig. 10 illustrates the relationship between the functional and structural phases of reticulo-endothelial and other mesenchymal derivative cells and the apparently

FIG. 10.—Diagram of cortisone-ascorbic acid interaction

of the abscissae. The apparent antagonism between ascorbic acid and cortisone supports the view that these two substances maintain the structure and function of the mesenchymal tissue *indirectly*. That the antagonism is not absolute, appears from several facts:

both substances are necessary for the development of hyaline (phase of healing); the effect of these substances on the mesenchyme seems to be dependent on the morphological phase of the mesenchymal cells *in loco* and to be related to Selye's "general adaptation syndrome"; the changes of the mesenchymal cells pass through the same phases of development; the degree of amyloid alteration in the negative phase seems to be determined not only by cortisone, but also by the degree of pyroninophilia in the preceding positive phase.

Probably most of the controversy regarding the effects of cortisone and ascorbic acid on the mesenchymal tissue may be explained by some such reciprocal mechanism of action.

As regards the development of amyloidosis, the effects of cortisone and ascorbic acid depend on the *condition of the tissues*, a fact that appears from the experiments. In an animal with pre-amyloid changes in an organ these will increase after treatment with cortisone, whereas other organs without such lesions may escape. The effect of cortisone on the mesenchymal tissue thus seems to depend on the local requirements for ascorbic acid and the possibilities of its utilization.

Mechanism of Development of Amyloidosis in Natural Disease

The experimental results may explain all types of amyloidosis in the natural diseases mentioned above, as it is evident that the conditions for amyloid-formation are present in all diseases where the regression of normal occurrence, or the development of hyalinos (inactive phase) after the preceding pyroninophilia (active phase), fails to appear. The amyloid phase may thus be considered a perverted

phase in the cellular synthesis of protein, in which the interaction between ascorbic acid and a hormone of the adrenal cortex similar to cortisone is decisive. All forms of typical (secondary) amyloidosis are analogous with the experimental amyloidosis after prolonged immunization or protein therapy, whereas the atypical (primary) amyloidosis may be explained by an imbalance in the reciprocal effects of the two substances. The different localization in primary amyloidosis is the same as that of hyalinosis in generalized sclerodermia (myocardium, tongue; and striated musculature), and transitional forms between sclerodermia and primary amyloidosis are also known (Jørgensen, 1944). Stoeber (1934) described cases of allergic conditions combined with so-called genuine amyloidosis, and Cazal (1942) mentioned a case of amyloidosis in a 7-year-old girl with *un état d'anaphylactique* as the only aetiologic factor.

The amyloidosis of rheumatoid disease differs from the usual secondary amyloidosis (in chronic suppurative conditions) in that it represents in itself an abnormal phase occurring under certain special conditions in the morphogenesis of the disease process. Actually there will be a difference only of degree between "amyloidosis in rheumatoid arthritis" and "primary so-called atypical amyloidosis" in which the abnormal phase of mesenchymal disorder has very recently developed.

Role of Mucopolysaccharide in Formation of Amyloid

Disturbances in the protein synthesis in mesenchymal cells are important in the formation of amyloid, but there may be other contributory factors. The amyloid substance is now generally considered to be a glycoprotein in which a mucopolysaccharide is attached to a globulin. The discovery of the hyaluronidase group of enzymes has renewed interest in the chemistry of glycoproteins—mucins, chondroproteins (Wright, 1950).

Disturbance in the extracellular matrix is of first importance in mesenchymal disorder. Hamilton and Syverton (1950), examining the spread of metachromasia in the myocardium of patients who had died of rheumatic fever, found a striking conformity with the occurrence of mast cells and concluded that the degranulation of tissue mast cells with the liberation of an acid-reacting mucopolysaccharide plays a part in the focal rheumatic process. The relation between mast cells and mucopolysaccharides has recently been studied in detail (Asboe-Hansen, 1950). Corticotropin and cortisone as well as ascorbic acid influence the occurrence of the metachromatic elementary substance; Schmith and Faber (1949) also found the hyaluronidase-inhibitory effect of serum diminished during treatment of rheumatoid arthritis with ACTH.

Since metachromasia of the extracellular matrix (and in certain cases granulated mesenchymal cells of the mast cell type) is a characteristic component in the active phase of mesenchymal disorder, and, like pyroninophilia, is controlled by the adrenal cortex-ascorbic acid interaction, it may be that a mucopolysaccharide or a modified product is attached to the proteins during the depression of the positive phase. It has been shown that the carbohydrate ester, chondroitin sulphuric acid, is capable of uniting firmly with several proteins to form stoichiometrically well-defined compounds (Meyer and others, 1937).

The study of pyroninophilic mesenchymal cells as precursors of hyalinosis and amyloidosis shows the significance of changes of *cellular* function and structure in mesenchymal disease as well as disturbance of the extracellular matrix.

Summary

(1) The mechanism by which cortisone maintains the structure and function of mesenchymal tissue is an *indirect* one, mediated by ascorbic acid.

(2) Several functional and structural changes of reticulo-endothelial and other mesenchymal derivative cells, including *amyloidosis*, are shown to be controlled by the *interaction* of cortisone-ascorbic acid.

(3) In mice, previously treated for several weeks with injections of casein, insufficient to produce amyloidosis, a few injections of cortisone promptly resulted in the appearance of amyloid in the spleen.

(4) The occurrence of *pyroninophilic mesenchymal cells* is considered to be a fundamental precursor in the pathogenesis of amyloidosis or hyalinosis.

(5) In experimental mesenchymal disorder, as well as in the natural diseases, three *typical phases* can be established:

- (a) *An active (positive) phase of pyroninophilia*, characterized by pyroninophilic mesenchymal cells in various organs and tissues, accumulation of plasma cells in the reticulo-endothelial system, elevated γ -globulin values in the serum, and, in many cases, metachromatic extra-cellular material.
- (b) *An inactive healing phase of hyalinosis*.
- (c) *A perverted (negative) phase of pre-amyloidosis or amyloidosis*.

Phase (a) is maintained by ascorbic acid and inhibited by cortisone; the continued administration of cortisone (with increased ascorbic acid deficit) may result in amyloidosis.

The established antagonistic effects of cortisone and ascorbic acid on mesenchymal tissue can be explained as an interaction of ascorbic acid and a hormone of the adrenal cortex of cortisone type controlling the phasic development in mesenchymal disorder.

(6) Thus the amyloid phase in rheumatoid arthritis and other conditions can be considered as a perverted phase in cellular synthesis of protein associated with a disturbance of some enzyme system.

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Action réciproque de la cortisone et de l'acide ascorbique dans la pathogénie de l'amyloïdose

RÉSUMÉ

(1) Le mécanisme par lequel la cortisone, hormone de l'écorce surrénale, maintient la structure et la fonction du tissu mésenchymateux, est *indirect*, par l'intermédiaire de l'acide ascorbique.

(2) On montre que plusieurs altérations fonctionnelles et structurales des cellules réticulo-endothéliales et d'autres cellules d'origine mésenchymateuse, comme celles qu'on rencontre dans l'*amyloïdose*, sont contrôlées par l'*action réciproque de la cortisone et de l'acide ascorbique*.

(3) Chez des souris traitées préalablement pendant plusieurs semaines par des injections de caséine, insuffisantes pour produire l'amyloïdose, celle-ci apparut rapidement dans la rate après quelques injections de cortisone.

(4) On considère que la présence de *cellules mésenchymateuses pyroninophiles* est un élément précurseur fondamental dans la pathogénie de l'amyloïdose ou de la hyalinose.

(5) Dans les troubles mésenchymateux expérimentaux ainsi que cliniques, on peut distinguer trois phases:

(a) Une phase pyroninophile active (positive), caractérisée par des cellules mésenchymateuses pyroninophiles dans de divers organes et tissus, par l'accumulation des cellules plasmatiques dans le système réticulo-endothélial, par le taux élevé de la γ -globuline dans le sérum et, dans beaucoup de cas, par la présence du matériel extra-cellulaire métachromatique.

(b) Une phase inactive de réparation (hyalinose).

(c) Une phase pervertie (négative), pré-amyloïde ou amyloïde.

La phase pyroninophile est maintenue par l'acide ascorbique et inhibée par la cortisone; l'administration continue de la cortisone—tandis que la pénurie d'acide ascorbique augmente—mène à l'amyloïdose.

L'effet antagoniste, bien prouvé, de la cortisone et de l'acide ascorbique sur les tissus mésenchymateux peut s'expliquer par l'action réciproque de l'acide ascorbique et d'une hormone de l'écorce surrénale du genre cortisone, contrôlant le développement phasique de trouble mésenchymateux.

(6) On peut donc considérer la phase amyloïde au cours de l'arthrite rhumatoïdale et des autres affections comme une phase pervertie de la synthèse cellulaire de la protéine, associée à un trouble de quelque système d'enzymes.

Acción recíproca de la cortisona y del ácido ascórbico en la patogenia de la amiloidosis

SUMARIO

(1) El mecanismo por el cual la cortisona, hormona de la corteza suprarrenal, mantiene la estructura y la función del tejido mesenquimatoso es *indirecto*, mediante el ácido ascórbico.

(2) Se demuestra que varias alteraciones funcionales y estructurales de las células réticulo-endotheliales y de otras células de origen mesenquimatoso, como las que se encuentran en *amiloidosis*, están controladas por la *acción recíproca de la cortisona y del ácido ascórbico*.

(3) En ratones, previamente tratados durante varias semanas por inyecciones de caseína, insuficientes para producir amiloidosis, ésta apareció rápidamente en el bazo después de pocas inyecciones de cortisona.

(4) Se considera que la presencia de las *células mesenquimatosas pironinófilas* es precursora fundamental en la patogenia de la amiloidosis y de la hialinosis.

(5) En disturbios mesenquimatosos experimentales así como en las enfermedades naturales se puede determinar tres *fases típicas*:

(a) *Fase pironinófila activa (positiva)*, caracterizada por células mesenquimatosas pironinófilas en varios órganos y tejidos, por una acumulación de las células plasmáticas en el sistema retículo-endotelial, por la cifra aumentada de la gama-globulina en el suero y, en muchos casos, por la presencia de material extra-celular metacromático.

(b) *Fase inactiva de reparo (hialinosis)*.

(c) *Fase pre-amiloide o amiloide, pervertida (negativa)*.

El ácido ascórbico mantiene la fase pironinófila y la cortisona la inhibe; la administración continua de cortisona—mientras crezca el déficit del ácido ascórbico—motiva una amiloidosis.

Los efectos antagónicos comprobados de la cortisona y del ácido ascórbico sobre el tejido mesenquimatoso pueden explicarse por la acción recíproca del ácido ascórbico y de una hormona de la corteza suprarrenal de tipo cortisona, controlando el desarrollo fásico del disturbio mesenquimatoso.

(6) Así pues, la fase amiloide en la artritis reumatoide y en otras afecciones puede considerarse como una fase pervertida de la síntesis celular de la proteína, asociada con un disturbio de algún sistema de enzimas.