THE RELATION OF THE VITAMINS TO THE REACTION INDUCED BY COAL TAR IN THE TISSUES OF ANIMALS.

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PLATES 7 TO 9.

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As the important problem in the study of cancer is the nature of conditions which regulate the normal growth of cells in the organism, it becomes necessary to study, in relation to tissue changes brought about by coal tar, the conditions and substances which promote normal growth. In previous papers (1) I have studied the action of coal tar in adult and embryonic tissues of the rat. These animals were fed on a balanced dietary throughout the experiments. Following the same procedure and technique, in so far as the introduction of coal tar into the tissue is concerned, I have modified the dietary in the feeding of a series of rats and studied the tissue changes produced by coal tar as affected by these conditions.

In the previous paper it had been shown that the chief effect of coal tar on the cells and organism is destructive rather than constructive. When drops of coal tar are injected into the subcutaneous tissue they attract the tissue cells to them from a wide area about them. As these cells leave the blood vessels and intercellular fibrils of the connective tissue the intercellular substances suffer not only a loss of their cells, but hyaline-like changes. The cells as they migrate to the edge of the tar round off, their nuclei and cytoplasm stain less sharply. Many of the first cells to arrive suffer a complete granular degeneration.

When a sufficient quantity of coal tar is injected into the subcutaneous tissue of an animal, the cells not only undergo this migration and show these degenerative changes, but the whole animal suffers a loss of weight, cachexia-like changes, and often an eventual death. The changes occurring in these animals as they are seen in the gross, are not unlike those that occur in animals with rapidly growing malignant tumors. Many of the earlier authors had already noted that lipoid solvents act to cause a death of the cells rather than any stimulation of them.

Such observations are seen in the articles by Bullock and Rohdenburg (2) and Champy (3). These authors had thought, therefore, that the action of these substances in the production of cancer is largely one of destruction with secondary regeneration. How the substances cause the degeneration and effect the general nutrition of the animal had not been determined, however.

The action of a single drop of coal tar is always limited. It acts to produce the changes noted above for only a short period of time. Its action is greatest at first, then it gradually wanes. It acts, therefore, in the tissue like a substance which dissolves some important constituent of the cell. Those cells which are drawn to the edge of these droplets of tar surround it as a continuous cellular layer one or many cells thick. After the tar has ceased to be active those of the cells which are not completely destroyed become active again. Their nuclei and cytoplasm stain more sharply. In the sparsely cellular tissue of adults a single injection of coal tar rarely ever draws more than a few cells to its periphery. In the more densely cellular mesenchyme of embryonic tissue and near the epidermis of adults many more cells are attracted. Where a large number of cells are crowded together about the tar they show growth and multiply as they recover from the primary effects of tar. Where only a few are attracted to the edge of the drop, they never show any growth, but slowly develop new intercellular substances and the whole eventually reverts to a hyaline scar.

The cells that move to the edge of the coal tar are the cells which are present in the tissues immediately about it. They are endothelial cells, fibroblasts, epithelial cells, leucocytes, and other wandering cells. With each new addition of coal tar more and more cells are attracted to the tar. With these repeated applications of tar the tissue about suffers much more exaggerated changes. Most or all of the cells in the nearby tissues are drawn to the tar. The surrounding tissues assume a hyaline appearance peculiar to tissue about actively growing cancers. In the more densely cellular masses accumulated by frequent applications of tar, growth and mitosis of cells intervene and cancer develops.

In studying these various effects of coal tar, it has been interesting to note that different animals respond differently to the injection of this substance. In a number of cases all the cells degenerated when they moved to the tar. In other cases they withstood the action of the tar and formed a dense cellular collar about each drop of this substance. Other authors have noted similar variations in these cellular responses in that cancer developed more rapidly in some of their animals, but no one has explained these variations. It became of interest, therefore, to investigate them in regard to the general state of nutrition of the animals and the diet they received.

For all these experiments young healthy actively growing animals have been used. The diets given them were modifications of those used by former workers on vitamins—Goldblatt (4), Gross (5), Zilva (6), Hume and Smith (7), Bond (8), Eddy (9), and McCollum (10). It was important to note carefully if disturbances in nutrition and the arrest or retardation of growth was due to vitamin deficiencies in these diets, or due to non-palatability, or decreased consumption of food. Results due merely to lack of food intake were discarded.

The chief immediate index to the effect of any particular diet is the gain or loss in weight of the animal. Repeated weighings were carried out during the course of the experiment. The injections of coal tar into the animal were regulated as to time and amount on the basis of the weight curves and the study of tissue changes, after determining the effect of similar quantities of tar in a series of animals fed on an ordinary normal diet.

Diets.

1. Balanced ration.
   - Egg albumin scales (finely ground) .... 25 gm.
   - Potato starch .... 40 "
   - Butter .... 15 "
   - Salt mixture (McCollum) .... 5 "
   - Lemon juice .... 5 cc.
   - Vegex* .... 5 gm.
2. Moderately high in vitamin A.
   - Egg albumin scales .... 25 gm.
   - Potato starch .... 40 "
   - Butter .... 25 "
   - Salt mixture .... 5 "
   - Lemon juice .... 5 cc.
   - Vegex .... 5 gm.

2, *a*. High in vitamin A.
   Cod liver oil, 5 cc., added to (2).

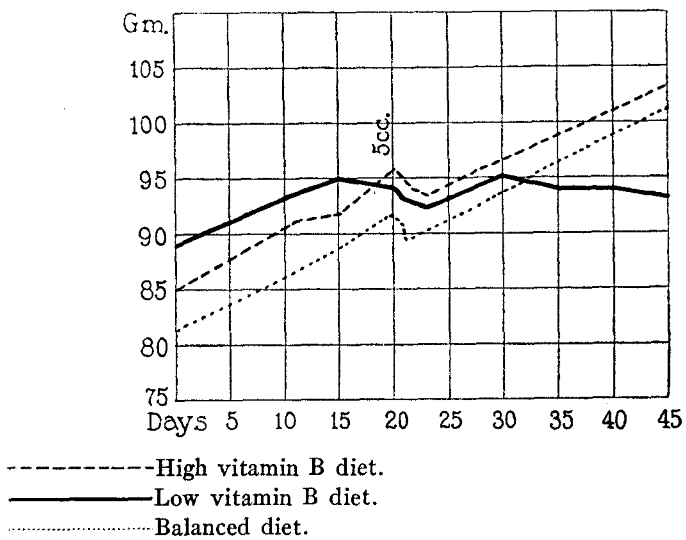
3. Deficient in vitamin A.
   Crisco substituted for butter in (2).
4. High in vitamin B.
   - Egg albumin scales .... 25 gm.
   - Potato starch .... 40 "
   - Butter .... 15 "
   - Salt mixture .... 5 "
   - Lemon juice .... 5 cc.
   - Vegex .... 10 gm.
5. Deficient in vitamin B.
   Vegex not included in the ration (4).
6. High in vitamin C.
   - Egg albumin scales .... 25 gm.
   - Potato starch .... 40 "
   - Butter .... 15 "
   - Salt mixture .... 5 "
   - Vegex .... 5 "
   - Lemon juice .... 10 cc.
7. Deficient in vitamin C.
   Lemon juice not added to the ration (6).

* Vegex is the source of vitamin B and a product furnished by the vitamin Food Company, Inc., Westfield, Mass.

EXPERIMENTAL DATA.

150 white rats were used in this experiment. They were divided into series, each series being made up of animals of practically the same age and weight. All were from the regular brother and sister strains of stock that has been bred in our laboratory during the past 5 years.

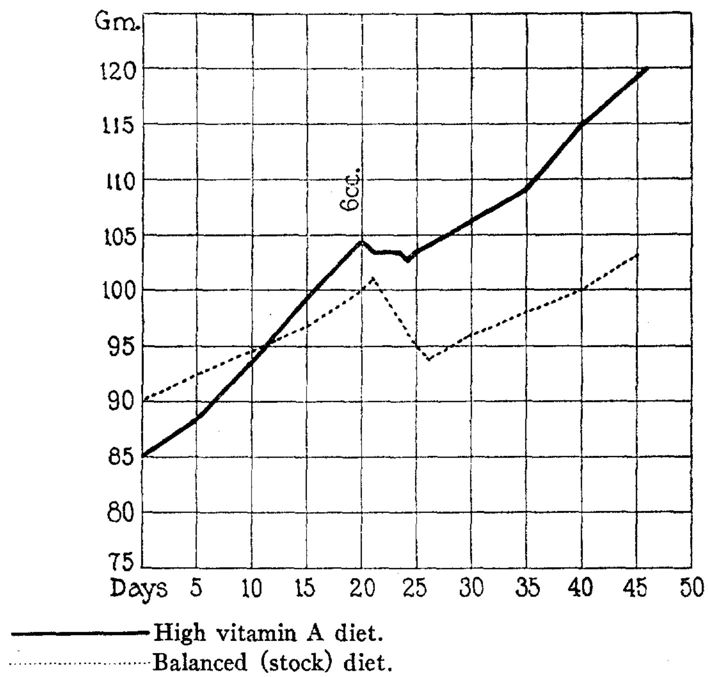
For the study of tissue changes around the particles of coal tar introduced under the skin, animals from each series were killed and autopsied at regular intervals of 2, 15, 30, 45, and 60 days after the injections of tar. A number of animals were kept for a longer period of time and some were autopsied and sections made of the tumor when the animal died as result of the deficiency diet. In other cases biopsies were made from animals under ether anesthesia. A certain number in each series will be kept for a long period of time and findings reported later.



TEXT-FIG. 1. Curve showing changes in weight before and after the injection of 5 cc. of coal tar into three rats on a high vitamin B, deficient vitamin B, and balanced diet respectively.

Discussion of Weight Curves.

The weight curves are practically self-explanatory and need emphasis only in regard to a few points of particular interest. Taking into consideration the weight of the animal and the amount of coal tar injected over a period of time, the weight curves of the animals on a deficient vitamin B and a high vitamin B diet are practically identical (Text-fig. 1). There is a definite decrease in weight after each injection of coal tar and the composite growth curve follows a line almost parallel to the base line. The animals on a balanced dietary lost

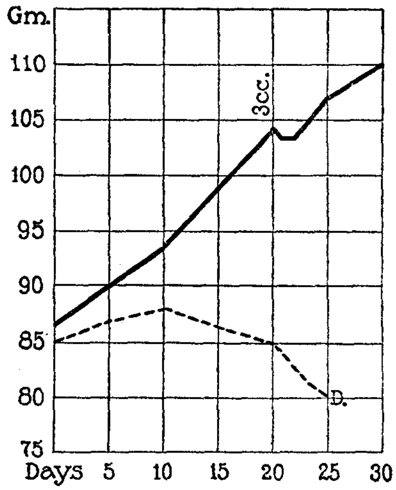


TEXT-FIG. 2. Curve showing changes in weight before and after the injection of 6 cc. of coal tar into two rats on a high vitamin A and a stock (balanced) diet respectively.

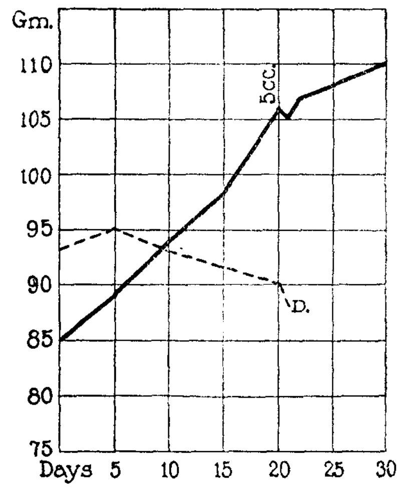
weight after each injection of coal tar, but they increased in weight gradually, not as rapidly, however, as an animal that has not been injected with coal tar (Text-fig. 2).

Animals fed the deficient vitamin A diet lost weight rapidly after the injection of coal tar, even after as small amount as 1 cc., and when the amount was increased to 3 cc., the animal died within a few days or even earlier. On the other hand, in the series receiving a high vitamin A ration the weight curve was little affected by the injections (Text-figs. 3 and 4). 10 cc. of coal tar did not always kill these animals.

A deficiency in vitamin C gives a weight curve similar to that of a balanced dietary. The withdrawal of this vitamin has no effect on the reaction of the animal to the tar.



Text-Fig. 3.



Text-Fig. 4.

——————High vitamin A diet.
– – – – – – –Low vitamin A diet.

Text-Fig. 3. Curve showing changes in weight before and after the injection of 3 cc. of coal tar into two rats on a high vitamin A and a low vitamin A diet respectively. Rats on a low vitamin A diet died (D.) quickly after injection.

Text-Fig. 4. Curve showing changes in weight before and after the injection of 5 cc. of coal tar into two rats on a high vitamin A and low vitamin A diet respectively.

Discussion of Tissue Changes.

In a previous paper (1) I discussed the tissue changes brought about by the introduction of coal tar into adult and embryonic tissue. The series of animals on the balanced ration dietary in this experiment presented tissue changes of the same character. There may be an earlier cellular degeneration and hyalinization in the tumors taken from the animals fed on the diet deficient in vitamin B but it is not marked. There is no cell activity after the 10 day period (Fig. 1).

In the animals fed on a high vitamin B diet, the collar of fixed tissue about the coal tar is more dense. Cells have been attracted from a wider zone than in the case of animals fed on a balanced ration or one deficient in vitamin B. Activity is not marked, but the cellular content of the collar is greater than can be accounted for by a mere drawing in of cells from the surrounding tissue. From 15 to 20 days after the injection of tar, degeneration and hyalinization have taken place (Fig. 2).

The striking feature of the tissue changes brought about in the deficient vitamin A animals is the sparcity of cells in the zone about the coal tar. The cells have been attracted from the surrounding tissue, but these cells have suffered a marked degeneration in their migration to the tar (Fig. 3). In only one case did they show even slight activity. All of them shrivel and break up at an early period.

In the animals fed on the normal diet of the laboratory, on the specially prepared balanced diet, and one deficient in vitamin A or B, or high in vitamin B, the coal tar tends to remain in large drops as it was injected into the tissue. It is quite different in this regard from the corn oil described by Burrows and Johnston (11). In most cases the corn oil breaks up quickly into numerous small droplets.

In the animals fed on a diet high in vitamin A this picture changes. The coal tar breaks up quickly into a large number of small droplets (Figs. 4 and 6). These are found scattered in a densely cellular tissue. In a few of the sections removed from animals fed a balanced ration the periphery of the drop of coal tar is partially broken up by the cells. The picture is quite different, however, from the complete breaking up of the drop in the animals fed a diet high in vitamin A. The cells about these droplets are large and contain well formed, sharply staining nuclei. Many of them are dividing by mitosis. This picture is most marked in the 15 to 30 day specimen (Fig. 5). After this period certain portions of the tissue surrounding the droplets undergo hyalinization, cellular activity continuing only in scattered cell nests. These cell nests present the picture of early sarcomatous changes. The collars of cells about the individual droplets are much larger than in animals fed on other diets. They are often thirty cells thick.

DISCUSSION.

In my previous paper (1) I had shown that coal tar acts on the organism to cause cachexia-like changes. Drops of coal tar placed in the tissue attract the fixed tissue cells to them from the surrounding tissues. They do not stimulate these cells to grow but the cells suffer partial or complete degeneration in this reaction. This action of the tar is not continuous, but limited to a short period of time. After the tar has ceased to be active those cells which have not degenerated completely recover from the action of the tar. Where the cells have become densely packed together about the tar active growth and division of these cells intervene and cancer develops. Where only a few cells are collected by the tar or only a few survive its action they form only a hyaline scar. There was no evidence that the tar acted, at any time, to stimulate the cells to grow. It acts as a simple solvent of lipoid elements in the cell. The degeneration in the cells and the cachexia may be the direct result of the removal of these lipoids by the tar.

Burrows (12) in his studies of body cells in the tissue culture has shown that these cells have many features in common with yeast and other unicellular organisms. They can grow independently as in cancer only when they are crowded together in a stagnant area where certain primary products of their metabolism can accumulate about them. They differ from many of the other unicellular organisms in that they have no special mechanism for migration. They migrate by liberating a surface tension-lowering substance absorbed only by proteins and fats. They can move only into proteins and towards larger droplets of fat which absorb this surface tension-lowering substance. The primary substance which must concentrate about these cells for them to become active is a water-soluble substance formed by them in proportion to the oxygen absorbed. It has been called the *archusia* (S). The surface tension-lowering substance has been called the *ergusia*. This latter substance is not liberated by the cell under all conditions, but only when the archusia (S) reaches a certain concentration (S_2). It is not re-formed in the cell until the archusia reaches a still higher concentration (S_3) or when the cells grow and divide. Coal tar dissolves the ergusia.

Body cells cannot retain the archusia. They can grow only when it is extracted from other sources and added to the medium or when they are crowded together in a stagnant medium from which it cannot escape. In the normal organism the archusia does not reach a concentration consistent for growth and re-formation of the ergusia. The question arose as to how these cells in the normal organism survive under such conditions. Is their mechanism for growth different from that in the culture, or do they obtain a supply of these substances from other sources? It seemed possible that certain of the vitamins were none other than these two essentials supplied by other growing animals and plants. To test this conception it became of interest to see the effect of coal tar on animals fed on a diet rich in one or the other of the various vitamins. These studies have shown that a diet rich in the water-soluble vitamin B stimulates the growth of these cells just as the archusia stimulates the cells in the culture, while the fat-soluble vitamin A protects the animal and the cells against the destructive action of coal tar or the loss of the ergusia to it.

In the light of these facts it has been possible, therefore, to draw the following conclusions.

SUMMARY AND CONCLUSIONS.

Drops of coal tar introduced into the subcutaneous tissue attract the fibroblasts, endothelial and other cells to them. These cells suffer degenerative changes through this action of the tar and the animal suffers cachectic-like changes and death from large doses of it introduced into the subcutaneous tissue. This action of the coal tar is limited to a short period of time, after which it becomes inert. The cells which have been drawn to it and which have not completely degenerated then slowly recover. Where large numbers of these cells are drawn to the tar they grow and divide after recovering from the initial effects of the tar. Cancer may develop. Where only a few cells are drawn to the tar they lay down intercellular fibrils and a scar eventually develops. Vitamin A fed in more than ample quantities to these animals protects the animals and the cells against the toxic action of the tar and stimulates and prolongs their secondary growth. Vitamin B stimulates the secondary growth of these cells. This action is limited in extent and time. It is followed by an early degeneration and hyalinization of the tissue.

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EXPLANATION OF PLATES.

The figures are photomicrographs of sections of tissue of adult rats, showing the cellular changes about drops of coal tar. Each series of animals was placed on the different diets 30 days before the injection of the tar or oil.

Plate 7.

Fig. 1. Moderately low power photomicrograph showing tissue changes 30 days after the injection of coal tar into the subcutaneous tissue of a rat fed on a diet deficient in vitamin B.

Fig. 2. Moderately low power photomicrograph showing tissue changes 30 days after the injection of coal tar into the subcutaneous tissue of a rat fed on a diet high in vitamin B.

Plate 8.

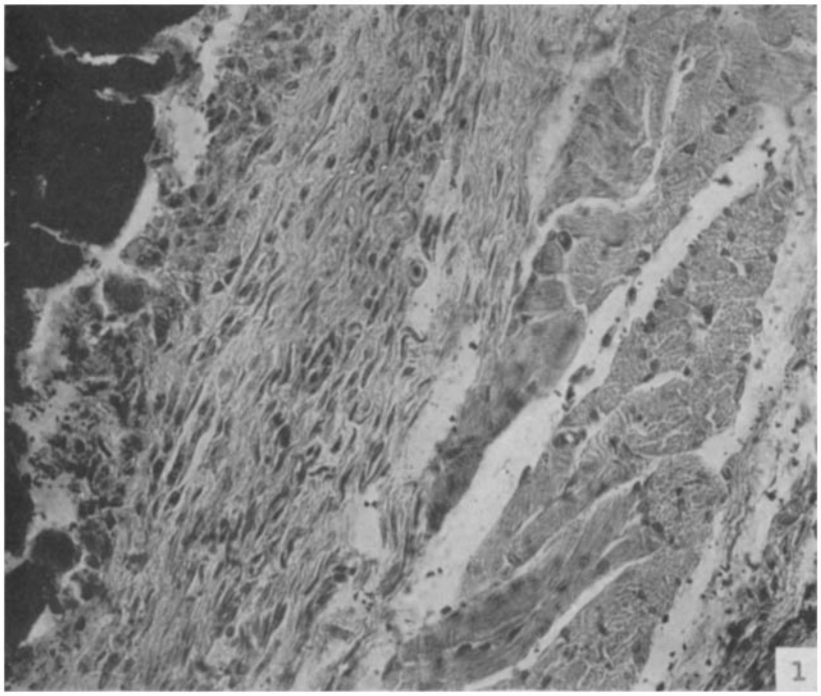
Fig. 3. Moderately low power photomicrograph showing tissue changes 28 days after the injection of coal tar into the subcutaneous tissue of a rat fed on a diet deficient in vitamin A.

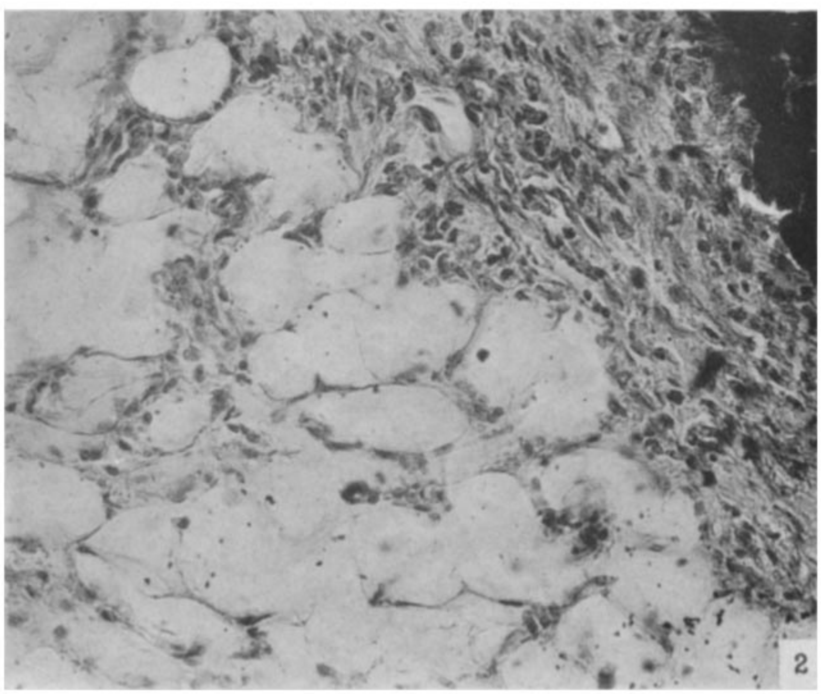
Fig. 4. Moderately high power photomicrograph showing tissue changes 30 days after the injection of coal tar into the subcutaneous tissue of a rat fed on a diet high in vitamin A.

Plate 9.

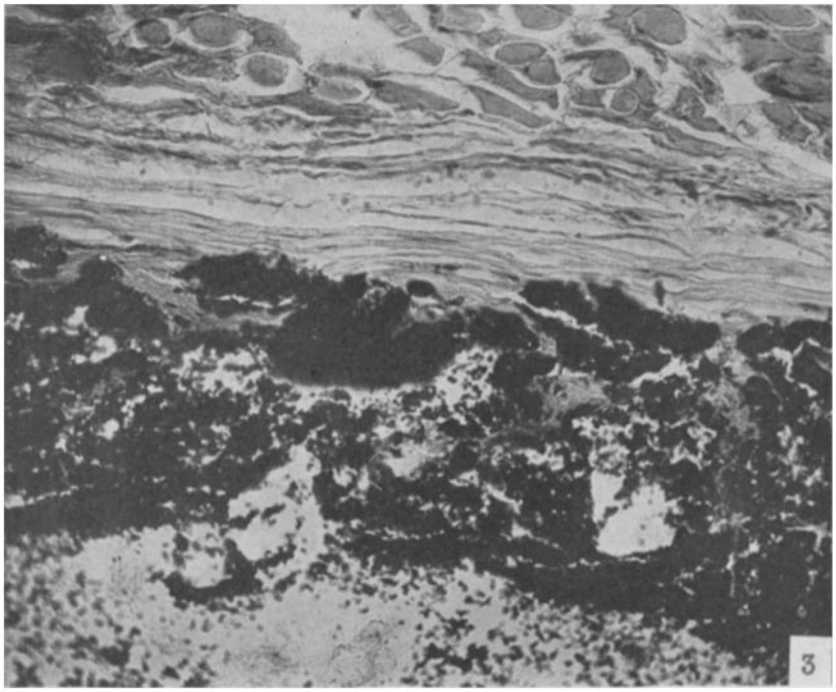
Fig. 5. A higher power photomicrograph of the same section as that shown in Fig. 4. The striking picture is the numerous mitotic figures as compared to sections taken from the rats on the other diets.

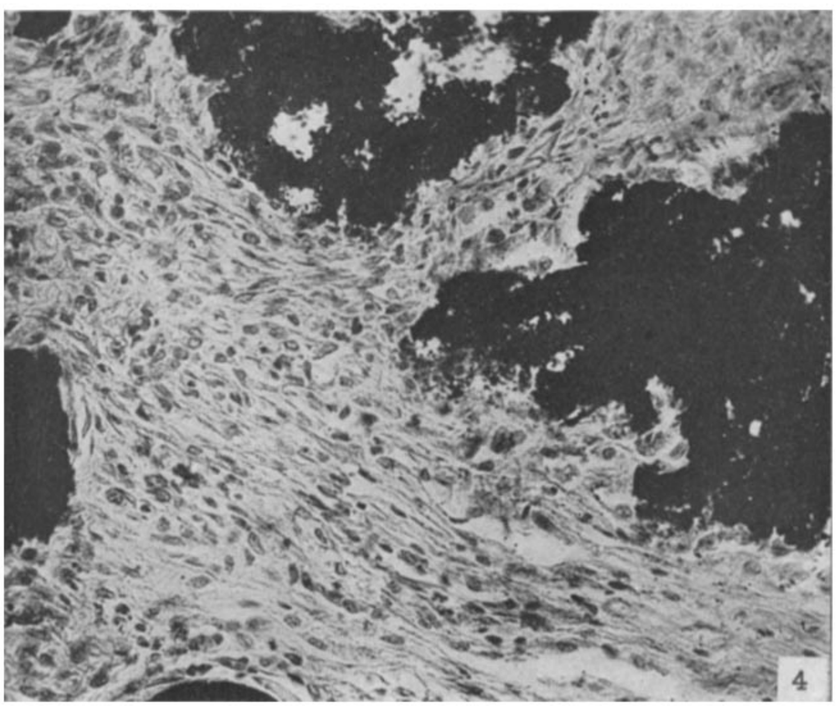
Fig. 6. A low power photomicrograph showing the breaking up of the coal tar into small droplets in the tissues of a rat on a high vitamin A diet. The section was taken from the rat 30 days after injection of the tar.





(Jorstad: Reaction induced by coal tar in tissues.)





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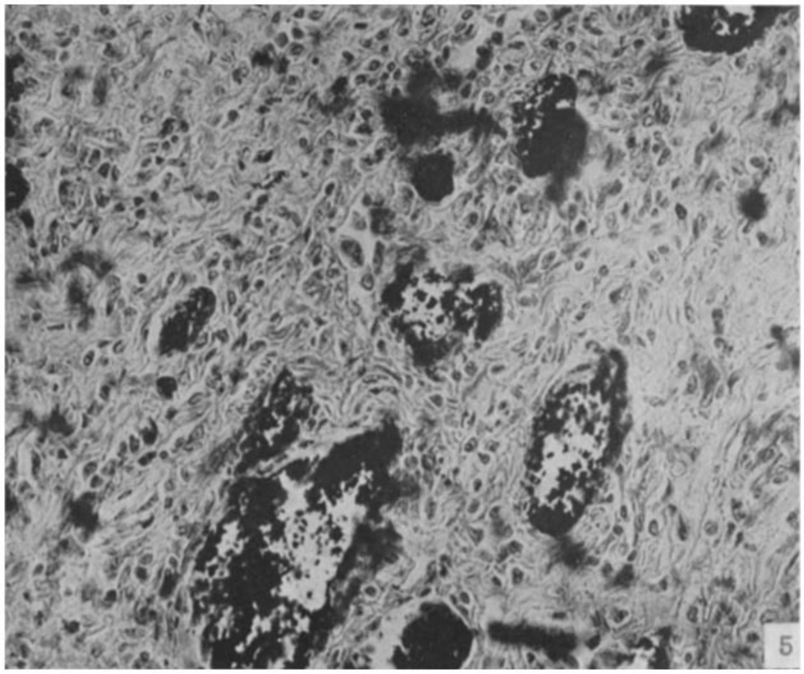

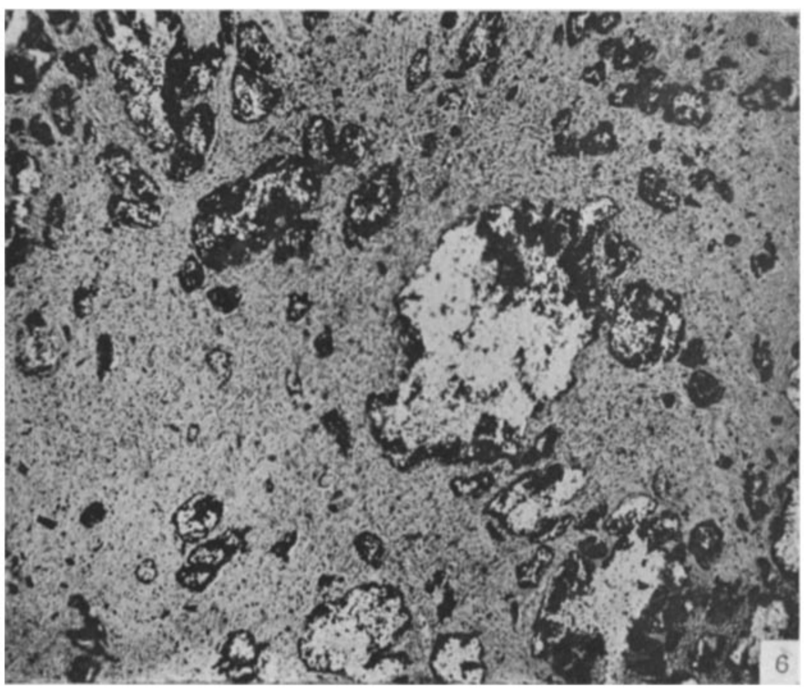

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