# STUDIES BASED ON A MALIGNANT TUMOR OF THE RABBIT.

## I. The Spontaneous Tumor and Associated Abnormalities.

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The frequency with which malignant tumors develop at the site of old syphilitic lesions or in association with long standing inflammatory processes of a syphilitic nature, has led many to believe that there may be some etiologic connection between syphilis and malignant disease. However, it is still uncertain whether syphilis, as such, can be regarded as a determinative factor in the incidence of malignant growths or whether its influence is confined to the realm of chronic irritation. Until recently, no experimental investigation of the relation existing between syphilis and malignant disease was possible, due to the lack of an animal in which both conditions could be studied experimentally. This obstacle has now been removed by the occurrence of a highly malignant tumor in a rabbit infected with syphilis, and the successful transplantation of the tumor to other rabbits, a brief report of which was made in March, 1921 (1).

The tumor has been under observation for more than 2 years and is now (November, 1922) in its twentieth generation. During this time, a careful study of the tumor has been made and it has been employed in an extensive series of experiments, the immediate object of which was to determine whether any essential connection could be established between syphilitic infection and the occurrence of malignant growths. The study of this problem has necessitated a collateral investigation of a wide range of conditions including circumstances affecting the growth or malignancy of the tumor, the effects of the tumor upon the animal organism, the influence of syphilitic infection upon resistance to tumor growth and upon an established tumor immunity, and the converse relationship of the effect

of tumor inoculation upon the course of a subsequent infection with *Treponema pallidum*; finally, through the joint use of the two experimental agencies (syphilis and tumor), controlling the one by the other, information has been acquired concerning the mechanism of animal resistance to syphilis as well as to the growth of the tumor.

So little is known of malignant tumors of the rabbit and of pathological conditions which may be associated with them, that it seems advisable to preface the reports of our experimental work by a detailed account of the tumor itself and of other abnormalities present in this animal.  In the descriptions which follow it may appear that too much space has been devoted to what might be regarded as uninteresting detail or conditions of little or no importance.  It seems well to state, therefore, that this treatment of the subject is intentional and is designed in part as a means of giving a comprehensive picture of the disease present in this animal, but its chief object is to place due emphasis upon a group of conditions which when properly correlated may prove to be of great value in interpreting phenomena of tumor growth.  In fact, as will appear in subsequent papers, a great deal of the experimental work which has been carried out with this tumor has been based upon an interpretation of the relation existing between these conditions and the origin and malignancy of the tumor.

Many benign tumors have been reported in rabbits, but malignant growths appear to be extremely rare and in only three instances has successful transplantation been accomplished (2, 3) and all these tumors appear to have been lost before any considerable experimental work could be carried out.  In our experience with rabbits, we have observed four instances of spontaneous tumors, three of which were in animals infected with *Treponema pallidum*.  Three of the four growths were adenomata of the kidney encountered in the course of routine autopsies.  The fourth tumor, which forms the subject of this paper, developed at the site of a scrotal chancre and was first noted 4 years and 3 months after inoculation.  The tumor is unique in that a complete history of the animal is available, including photographic records of the syphilitic lesions, from the time of inoculation with *Treponema pallidum* to the occurrence of the tumor, with a subsequent history covering the progress of the growth over a period of 6 months.  The essential features of this history will be summarized as briefly as possible.

## Clinical History.

The animal was a male albino of a stocky build, weighing 1,900 gm. and fully developed when first placed under observation.

*Inoculation and Development of Scrotal Lesions.*—On June 11, 1916, the animal was inoculated in both scrota with the Zinsser-Hopkins strain of *Treponema pallidum* by the subcutaneous implantation of a bit of infected testicular tissue. Chancres developed in both scrota but completely disappeared during the summer.[1] 5 months after inoculation, there was a recurrence of the lesion in the left scrotum which assumed the form of a circumscribed but rather irregular nodular mass involving the deeper layers of the skin and subcutaneous tissue. This nodule increased slowly, reaching a maximum size of 1.9 by 1.5 by 1.8 cm. at the end of $3\frac{1}{2}$ months. It then began to soften at the center and resolution took place without perforation of the skin. Resolution was slow and interrupted by intervals of slight renewal of activity. 18 months after inoculation, a small opaque mass was still present in the scrotum. Meantime, lesions had developed in both testicles with the production of a slight fibrous thickening and some atrophy which was more marked on the left than on the right.

*Late Secondary Lesions.*—Nothing more of importance was noted until April, 1919 (2 years and 10 months after inoculation), when a series of cutaneous lesions developed along the outer sides of both hind feet and disappeared within a few weeks. These lesions presented the appearance of small oval or flattened papules with scaly surfaces. On June 10, there was a slight iritis in the right eye, and in November, there was a recurrence of the cutaneous lesions on the feet.

*Development of Chronic Inflammatory Reaction in the Left Scrotum.*—In January, 1920, a chronic inflammatory condition developed in the left scrotum which probably marked the beginning of the series of local changes that culminated in the development of the tumor. The condition present is shown in Fig. 1, and may be described as a diffuse infiltrative process producing a parchment-like thickening of the skin over a large part of the ventral surface of the scrotum with desquamation and exfoliation of the epithelium over the center of the lesion. At first, the affected area was pale and translucent with a smooth and glistening surface. As the condition progressed, delicate silvery scales began to form and eventually the central part of the lesion became covered by thin crusts, the removal of which left moist or weeping patches. An attempt to demonstrate spirochetes in the lesions by dark-field examination gave negative results. The subsequent history of this affection was one of improvement or complete recovery followed by relapse.

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[1] Seasonal conditions exercise a marked influence upon the course of syphilitic infections in the rabbit. During the summer months, there is rarely the same degree of regularity in the course of the disease as at other seasons, while the early lesions tend to be slight and of short duration followed by relapse during the fall or winter months.

*Papillomatous Affection of the Lips.*—In March of the same year, small patches of a peculiar papillomatous growth appeared on the skin surface of the left upper lip near the midline, over the chin, and on the right lower lip toward the angle of the mouth. The growth was composed of soft thread-like processes of a grayish white or pale yellow color averaging about 2 to 3 mm. in length. They were firmly united to the skin and reminded one of soft stubby hairs, markedly enlarged. This condition persisted for some time with no tendency to spread but had almost disappeared at the time the animal was killed.

*Demonstration of the Persistence of the Syphilitic Infection and Appearance of a Tumor Nodule in the Scrotum.*—In September, 1920, there were no lesions visible, except an area of slight infiltration in the left scrotum, the surface of which was covered with fine scales. At this time, test inoculations made from a popliteal node showed that the animal still harbored viable treponemata (4).[2] On October 1, a small, translucent nodule with smooth and glistening surface was noted in the left scrotum; its position corresponded with that of the primary syphilitic lesion and with the upper portion of the area previously affected by the eczematoid condition described. This nodule grew rather rapidly but irregularly, and at one time, following aspiration with a needle, diminished greatly in size. On October 18, the nodule measured 1 by 0.8 by 0.2 to 0.3 cm. The appearance and mode of growth of the lesion closely resembled those of an active syphilitic granuloma, but several attempts to demonstrate spirochetes by dark-field examination gave negative results; hence, it was excised under ether anesthesia for histological examination. The appearance presented at this time is shown in Fig. 2. This lesion will be referred to subsequently as the primary tumor. At this time, the animal weighed 2,500 gm. and was apparently in excellent physical condition, except for the presence of a peculiar diffuse thickening of the skin, the history of which may be given at this point.

*Nodular and Diffuse Induration of the Skin.*—Several months before the development of the tumor nodule in the scrotum, it was noted that the skin of the animal was becoming unusually thick, especially in the region of the shoulder girdle where we were accustomed to grasp the animal in removing him from the cage. No especial significance was attached to this condition until the fall of 1920 when the thickening became extremely marked and almost board-like over the region of the right shoulder (the area covered by the fingers in the act of grasping). The development of this condition corresponded roughly with the occurrence of the growth in the scrotum. In November, 1920, a number of small, sharply circumscribed and intensely indurated nodules were found in the skin over the back, the sides of the thorax, and in the cutaneous folds of the flanks. These nodules were imbedded in the deeper layers of the skin; in general, the overlying skin was pale with an irregular and scanty growth of hair, while in places the surface was smooth and glistening. Associated with these lesions, there were irregular areas

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[2] Brown and Pearce (4), p. 3, Rabbit 1.

of thickening in the skin such as occur during the shedding period. Some of the nodules ultimately disappeared, while others persisted until the death of the animal.

*Reinoculation and Cross-Inoculation.*—On October 28, 1920, the animal was reinoculated in the right testicle and on the sheath with the strain of *Treponema pallidum* recovered from its own popliteal lymph node. The reaction in the skin was very slight but was decidedly more marked in the testicle, lasting for 11 days before it began to recede. On November 15, a cross-inoculation with the Nichols Strain V was done in the skin at the base of the right ear and on the sheath. The reaction on the sheath was again slight but that on the ear was quite marked and assumed the form of a spreading infiltration with a scaly surface which persisted for 10 days and then gradually disappeared.

*First Tumor Recurrence.*—After excision of the primary growth, the wound in the scrotum healed quickly, leaving a thin and delicate scar. 1 month later, a tiny flattened nodule, identical with the one described above, appeared at the lower end of the scar, but soon disappeared. On December 28 (2½ months after excision of the primary growth), a second nodule was noted in the scrotum well above the area of the scar. This nodule grew rapidly with the formation of a superficial ulcer covered by a reddish brown crust as shown in Fig. 3. With the appearance of this lesion, the left inguinal nodes began to enlarge, reaching the condition seen in Fig. 3. A search for spirochetes in the cutaneous lesion was again made but with negative results. On January 13, 1921, the nodule in the skin and the nearest lymph node were removed under ether anesthesia (first recurrence).

*Second Recurrence.*—The wound healed slowly after this operation; a sinus formed which discharged a clear amber-colored and slightly oily fluid, but there was no suppuration. The upper lymph node continued to enlarge and there was an almost immediate recurrence of the growth in the skin. The process now assumed a more diffuse character and quickly extended across the midline in the pubic region; the nodes in the right groin began to enlarge and from this time onward there was a gradual loss of weight and strength.

*Increased Activity of Growth and Physical Deterioration.*—During February, there was a decided increase in the activity of the growth, which spread diffusely but irregularly over the pubic and lower abdominal regions, and by March 1, there was a decided deterioration in the physical condition of the animal. In addition to a slight loss of weight, and general weakness, there were marked weakness of the hind quarters, slight diarrhea, and relaxation of the anal and vesicle sphincters with dribbling of urine; the anus and sheath were swollen and reddened, and there was beginning ulceration along the mucocutaneous margins; the pupils were small (4 to 6 mm. in dim light) and irregular, and reacted feebly to light (flash) (spinal miosis.)

*Terminal Condition.*—By March 10, the animal was in extremely poor physical condition and was killed with ether. At this time there were extreme weakness and a loss of weight amounting to 300 gm.; the wounds were all clean but there was only slight union; the necrosis about the anus and sheath was spreading but the

tissues were comparatively dry and there was no appreciable inflammatory reaction; there was complete loss of sphincter control and movements of the hind legs were feeble, spastic, and incoordinate. Examination of the blood gave the following results.

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| Red blood corpuscles | 3,040,000 |
| White blood corpuscles | 38,000 |
| Hemoglobin, Sahli | 80 per cent. |
| Palmer | 60 per cent. |

Differential count.

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| Polymorphonuclear leucocytes | 55 per cent. |
| Basophils | 1 per cent. |
| Small mononuclears | 20 per cent. |
| Large mononuclears and transitionals | 24 per cent. |

A few normoblasts were seen.

*Transplantation.*—Three attempts were made to transplant the growth to other rabbits. The first attempt was unsuccessful, but by the use of intratesticular inoculations, a good growth was obtained in both the second and third series of animals and subsequent transfers by this method have almost invariably given 100 per cent takes.

## Pathology of the Excised Lesions.

Before proceeding with the report of the postmortem examination, which revealed the presence of a variety of conditions other than tumor metastases, it seems best to give a description of the lesions which were excised during life. For routine examination, these tissues, as well as others to be described, were fixed in Zenker's fluid and in formalin and stained with hematoxylin and eosin or with methylene blue and eosin.

## Primary Growth.

The primary growth appeared as a small oval mass imbedded in the skin. It was firm in consistency, of a pale grayish white color, comparatively translucent, and fairly well demarcated from the surrounding tissue. Microscopically, this mass was composed of two distinct nodules (A and B, Fig. 4) separated from one another by a smaller nodule (C) which was continuous above with a narrow zone of growth in the papillary layer of the skin.

The largest and apparently the oldest of the nodules (A, Fig. 4) was located in the papillary layer of the skin and was surrounded on the lateral and lower surfaces by thin but compact bands of fibrous connective tissue (Fig. 4). This portion of the growth was made up of cells of an epithelioid type whose appearance and

arrangement are shown in Fig. 5 which was taken from the area designated as *a* in Fig. 4. Toward the periphery, the cells diminished in size, were round or oval in shape, and stained more intensely with hematoxylin.

The nodule as a whole was of a compact structure with only a slight connective tissue stroma and comparatively few blood vessels or lymph spaces. There were no areas of necrosis and very few cells showed mitotic division.

Toward the skin surface, the tissue was more loosely arranged than elsewhere, and as the epidermis was approached, gradually assumed the character of granulation tissue containing scattered islands and strands of epithelioid cells resembling those toward the center of the nodule (Figs. 6 and 7 from *b*, Fig. 4). This portion of the growth contained numerous dilated lymph spaces and thin walled vessels many of which showed a loose perivascular accumulation of lymphocytes. Capillary vessels were especially numerous immediately below the epidermis and in places appeared to invade the epithelial layer. At these places, there were numerous open spaces, some of which extended into the epidermis, as in Fig. 6, and were partly or completely filled with blood or with loose masses of epithelial cells, some of which were in process of division. In other instances, the appearance was that of an intracystic papilloma (Fig. 7). Many of these lesions bore a striking resemblance to those seen in Paget's disease. In this zone, there were also a number of irregular downgrowths from the surface epithelium, and these became more numerous toward the periphery, where they were continuous with a series of large papillary processes in the surrounding skin area (Fig. 4, left). Hair follicles were present in only a few places and these rarely extended to the surface. As a rule, they were atypical in appearance and grouped in nests with a slight zone of inflammatory reaction about them. There was unusually active mitotic division among the cells composing these follicles but little or no tendency to differentiation into the cortical substance of the hair.

Another feature of this nodule was the presence of scattered nests, or whorls, of cells resembling those of the surface epithelium (Fig. 5) and of multinucleated giant cells apparently composed of partially keratinized or degenerated cells.

Finally, the reaction about the deeper surfaces of the nodule was extremely slight; the connective tissue of the corium was reflected about it in the form of a capsule but there was very little cellular reaction.

The second nodule of the main tumor mass (*B*, Fig. 4) appeared as a bulbous expansion in the deeper layers of the corium, continuous with a smaller central nodule (*C*, Fig. 4) and with that portion of the growth lying immediately below the epidermis. It was partially enclosed on all sides by compact bands of fibrous connective tissue derived from the corium, while similar strands of tissue extended into the depths of the nodule. This part of the growth was composed of irregular round or oval cells with a granular or vacuolated cytoplasm, the outlines of which tended to be ragged and ill defined. Mitotic figures were numerous in all parts of the section as shown in Figs. 8 to 10 (from *c* and *e* of Fig. 4).

The structure of the growth also differed from that of the older nodule in that the cells were arranged in the form of a loose network enclosing irregular spaces or

vacuoles which apparently contained fluid or semifluid material of some kind. This produced a spongy appearance which can be recognized even in Fig. 4. The stroma consisted of clearly defined strands of connective tissue which followed the course of the larger vessels with no distinct network of intercellular fibrils (Figs. 8 and 9). There were numerous capillary vessels in all parts of the growth, with a system of prominent lymph spaces toward the periphery (Fig. 10).

The line of separation between the tumor cells and the connective tissue of the skin was not clearly defined. In places, there was a thick wall of fibrous tissue of almost normal appearance with very little cellular reaction (d, Fig. 4). At other points, the capsule was invaded for some distance by narrow columns of cells or was broken up into irregular strands by heavier masses of tumor growth. This condition was evident toward the upper surface, where columns of cells extended downward from the epidermis and joined with the mass in the corium (Fig. 4), but the break in the connective tissue was even more complete where nodules B and C united.

The extension of the growth in these directions also served to establish a connection with that part of the growth which was situated immediately below the skin surface. As shown in Fig. 4, the epidermis in general was greatly thickened and there were numerous irregular downgrowths of epithelium. These changes were associated with an active proliferation of the epithelial cells and in many places there was an outgrowth of cells which filled the intervening spaces of the papillary layer, as is indicated by the fading outlines of the process marked by an arrow in Fig. 10. This picture is characteristic of the condition present over the greater part of this area and is of considerable importance in tracing the origin of the tumor. As a rule, the growth of these cells was restricted to the loose connective tissue zone of the outer layer of the skin, but in a few places, as in Fig. 10, the cells penetrated to a greater depth. There was also a tendency to invade the lymphatics, and numerous tongue-like projections or masses of cells were found free in the lymph spaces of the tumor itself or in adjacent parts of the skin (Fig. 11 from f, Fig. 4).

It is also important to note that as the growth extended downward the appearance of the cells tended to change in one of two directions. Where there was obstruction to further growth, as along the line of contact with the dense fibrous layers of the corium, the cells became smaller, resembling the so called basal cells of the human epidermis, as may be seen in Fig. 12. Where less resistance was encountered, a transition to a more active type of cell occurred, as seen in Fig. 10, in which the growth has penetrated into the depths of the corium, forming the nodular mass previously described (B),

The remainder of the main tumor growth appeared as an irregular pyramidal mass (C, Fig. 4), wedged in between the two larger nodules A and B. An examination of a series of sections showed that this nodule might be regarded either as a downward extension of the growth in the papillary layer or as a part of nodule B. It was composed largely of cells of the type described in nodule B with a gradual transition toward the periphery into smaller cells of the basal type. In places,

these cells tended to be grouped about the lymphatics. Here again there were numerous polynucleated giant cell masses in the upper portion of the growth which appeared to be of epithelial origin as in the case of nodule *A*.

The skin about the central mass of tumor growth showed an interesting series of abnormalities which are believed to have a bearing upon the origin of the tumor. In general, there was a decided thickening of the epidermis with the development of large and irregular papillary processes. The cells were swollen and granular and many of them vacuolated, while mitotic figures were numerous in all parts of the epithelium.

The papillary layer of the skin was the seat of a low grade chronic inflammatory reaction which was peculiar in that the growth of capillary vessels was proportionately greater than that of the fibroblasts or the cellular infiltration. This reaction was present in all parts of the section but was more marked in some places than in others. The deeper layers of the skin were also abnormal. The individual fibers of connective tissue were distinctly swollen and irregular in outline and were collected into coarse bundles forming an irregular network or compact layers of highly refractile tissue which stained intensely with eosin. In places, there was an increase in the thickness of this portion of the skin. The walls of some of the vessels were also thickened and there were scattered patches of lymphocytic infiltration, some of which showed a perivascular arrangement.

There were practically no hairs projecting from this part of the skin and very few hair follicles were present. Some of these showed no appreciable abnormality, while others were in a state of active and somewhat atypical hyperplasia.

The most striking condition, however, was the presence of numerous areas of active and atypical epithelial growth which varied from a moderate hyperplasia to conditions such as those illustrated in Figs. 12 and 13 which are taken from different parts of the skin lying anterior to the area of the primary growth (*g*, Fig. 4). The majority of these lesions appeared to develop as downgrowths from hyperplastic papillary processes and tended to retain this form and structure as in Fig. 12. In other instances, the growth was more diffuse; the cells appeared to arise from a wider area and the outlines of processes tended to be obliterated. A third type of growth was that shown in Fig. 13, which is suggestive of a hair follicle, even the cross-sections presenting something of the appearance of a bulb, and occasionally, a clearly defined hair follicle formed the central structure in one of the lesions.

Other features of interest in these lesions are the variety of cells present, the transition from one type of cell to another, and the tendency in places to a perivascular arrangement, especially evident in the case of the grouping of the smaller cells about lymph spaces. It should also be noted that none of the lesions showed a tendency to prickle cell formation, and only one small area of keratinization was found in the entire area represented by the sections studied.

The downgrowth of epithelium in lesions of this type was usually arrested at the surface of the fibrous layer of the corium, as is clearly indicated in Fig. 12. In exceptional instances, however, the growth extended deeper as was the case with that in Fig. 13. The reaction about the lesions varied somewhat, but on the

whole, the growth of fibroblasts and cellular infiltration were less marked than is usual in simple chronic inflammatory conditions of the skin or in syphilitic infections, while the development of capillary vessels was nearer normal.

In portions of the skin still further removed from the primary growth, there were similar but less pronounced changes in both the epidermis and the subjacent tissues, the condition usually present being essentially that shown in Fig. 14.

## Cutaneous Recurrences.

The first cutaneous recurrence consisted of a single large nodule with an ulcerated surface situated in the papillary layers of the skin and a number of smaller nodules imbedded in the fibrous layers.   The structure of these lesions was almost identical with that of the most actively growing part of the primary tumor designated as nodule B (B, Fig. 4), except that the cells were even more irregular and less clearly defined and were much more loosely arranged.   The growth was also more diffuse, and while in some places there was still a fair degree of separation between tumor and connective tissue, the blending of the two had become more complete; there was still a notable deficiency in the cellular reaction, and small columns and nests of tumor cells were widely distributed through adjacent parts of the corium.

In addition to the lesions described, the epithelium of the surrounding skin still exhibited an atypical hyperplasia.   In some respects, the growth of epithelium was the same as that seen in wound healing or in many chronic inflammatory processes, but in places (Fig. 15), it was strongly suggestive of a primary malignant growth, the cells and character of the growth resembling that part of the primary tumor represented in Fig. 10.   In other places, the most striking condition was an abnormal growth of hair follicles which resulted in the formation of giant masses such as that shown in Fig. 16.   The cells of the mass were in a state of active division, and although hairs were developed, they were malformed and comparatively few of them appeared to reach the surface.   In deeper parts of the skin, bulb-like structures were numerous, the majority being unusually large and containing many cells in process of division.

The second skin recurrence contained only a few small nodules analogous to those of the first, but was composed largely of a diffuse growth practically identical with that in Fig. 15 which appeared to arise directly from the surface epithelium and in places extended through the entire thickness of the skin with little or no reaction in the surrounding tissues.

The structural resemblance between successive cutaneous lesions is sufficiently close to identify them as parts of a common process; hence, the so called recurrent growths might be regarded either as direct extensions or as metastases from the original growth in the scrotum.   Upon this basis, however, it would be difficult to explain the connection

which appeared to exist between the epidermis and the growth in the deeper layers of the skin in places far removed from the primary tumor. This feature of the lesions is more suggestive of the extension of some underlying condition or state such as favored the development of the primary tumor than of an extension of the tumor itself, a suggestion which is strengthened by the presence of a typical epithelial proliferations of a similar character in other parts of the body. These will be described later.

### Metastases in the Inguinal Lymphatics.

The lymph nodes removed from the inguinal regions at the second and third operations were greatly enlarged, the capsules being thickened and of a dense fibrous consistency as shown in Fig. 17. The interior of the node was composed of a soft pasty material, somewhat slimy or cheesy in consistency, which tended to separate from the capsule. The color varied from an opalescent white to a grayish pink interspersed with areas of yellowish gray. In water, this material formed a flocculent suspension with only a few strands of fibrous tissue.

Microscopically, the parenchyma of the nodes was entirely replaced by a growth composed of cells of two main types, the character varying according to their distribution (Fig. 17). The cells toward the center of the growth were oval, rounded, or spindle-shaped with a granular and slightly basophilic cytoplasm. Toward the periphery, these were replaced by larger cells with a reticulated or even vacuolated cytoplasm which stained a pale pink or lilac color. Cells of this type were more numerous in the right inguinals than in the left, the growth on this side (right) being of more recent origin and more active, although mitotic figures were extremely numerous in all parts of both nodes. The framework of the lesions consisted chiefly of thin walled vessels some of which were distended with blood. These vessels were in striking contrast with those in the capsule and surrounding tissue which frequently showed great thickening of the walls and a narrow lumen. The connective tissue present was confined largely to the region of the vessels.

In the inguinal nodes, extensive areas of necrosis were seen for the first time (Fig. 17), associated with a distinct perivascular arrangement of the living cells, especially noticeable toward the center of the node.

Although the capsule of the nodes was greatly thickened, the connective tissue showed a distinct hyaline appearance with swelling or fragmentation of the fibers and was invaded by the tumor cells on all sides.

### Autopsy.

At autopsy a variety of pathological conditions was found. In addition to metastatic growths in many organs, there were obvious

abnormalities of the skin, the tongue and buccal mucosa and esophagus, the cardiovascular system, the lymphoid tissues, the bones, the testicles, the thymus, the thyroid, the suprarenals, and the pituitary gland.   For convenience, these conditions may be divided into two groups, the first including tumor metastases and associated reactions in affected organs, and the second, conditions which appear to have had some connection with the presence of the tumor or with the syphilitic infection or with both.

### Metastases and Changes in the Affected Organs.

The organs or tissues in which tumor metastases were found included the lungs, the liver, the kidneys, the spleen, the bones, and the inguinal, the pelvic, and the retroperitoneal lymph nodes, and the inguinal fat.   The general characteristics of the lesions were much the same in all organs, but the growth was most abundant in the liver, the spleen, and the bone marrow, while the liver contained the greatest variety of lesions.   The liver will serve, therefore, as a basis for a general description of the tumor growth and the associated tissue reaction.

Liver.—The liver was approximately normal in size and of a yellowish brown color and contained numerous round or oval masses of a milky white or opalescent appearance, which varied from minute points to upwards of 1.5 cm. in diameter (Fig. 18).   Most of these masses were sharply demarcated and of a firm elastic consistency, but the more translucent ones were soft and flaky and somewhat slimy to the touch.   There was a prominent network of distended vessels about some of the nodules and a few showed small petechial hemorrhages or grayish yellow foci of necrosis.   On the whole, however, the appearance was comparatively uniform, the chief difference being the degree of translucency or opacity. The liver was free from coccidiosis and no cysts were found in the abdominal cavity.

Histologically, there were innumerable areas of tumor growth ranging from a collection of a few cells in the capillary spaces with no reaction in the surrounding tissues to large nodular masses enveloped by a thick wall of connective tissue. Examples of these lesions are given in Figs. 19 to 21.   The early lesions were composed of irregular round or oval cells with a granular and slightly basophilic cytoplasm.   In most instances, these cells formed compact masses or solid cords which extended along the capillary spaces.   Occasionally, however, the cells showed a radial arrangement about a central space containing albuminous material which suggested the appearance presented by a section of the deeper portions of a hair follicle.

The cells of older lesions differed greatly in appearance. The prevailing types were those seen in Figs. 19 to 21. Some nodules were composed entirely of small round or oval cells, as in Fig. 19, while others were made up of large, irregular, vacuolated cells such as those in Fig. 20. More often, there was an intimate combination of a variety of cells, as in Fig. 21, with the production of a lesion analogous to that of the primary tumor or a transition from one type of growth to another in different parts of the same nodule.

The structure of the growth in the liver was also subject to considerable variation. In general, the cells were arranged in the form of spongy or compact masses with comparatively little stroma, which was distributed chiefly along the course of the blood vessels. Many of the lesions preserved this structure throughout, while others showed a tendency to the formation of an irregular network of spaces toward the center of the nodule which were filled with a fluid or semifluid material. No intercellular network of connective tissue fibers could be demonstrated and only a few minute droplets of lipoid material could be brought out in the tumor cells by staining with Scharlach R.

The blood supply of some lesions was fairly abundant and there was frequently a tendency to a perivascular arrangement of the tumor cells. In such cases, the growth was usually composed of small round or oval cells with a granular cytoplasm. On the other hand, nodules composed of irregular, vacuolated cells contained fewer vessels as a rule and in extreme cases were practically devoid of a capillary network, the few vessels present being confined to stroma lines. This suggests that nutritive conditions were in part responsible for the cellular variations and that the vacuolation of cells was due to colloid or mucoid degeneration. This is borne out by the physical characteristics of these lesions (soft and slimy).[3]

Mass necrosis was present in only a few of the larger nodules and was confined to the center of the growth. Focal hemorrhages occurred under similar conditions.

The reaction about the liver metastases consisted chiefly in the development of connective tissue with very little cellular infiltration. The extent of this growth was as variable as that of the tumor itself. In fact, character of growth and extent of reaction appeared to be closely related. At one extreme, there was a group of lesions composed of small round or oval cells surrounded by a thick wall of fibrous connective tissue invaded to a greater or less extent by tumor cells. Fig. 19 was taken from the outer edge of a lesion of this type. At the other extreme, there were nodules, almost if not quite as large, made up of large vacuolated cells which showed little or no tendency to encapsulation, as seen in Fig. 20, and these two conditions were frequently found in close apposition to each other.

In any event, there were only a few instances of a clearly defined zone of connective tissue beyond which the tumor cells had not penetrated. As a rule, the surrounding connective tissue was diffusely invaded (Fig. 19) or was split up into a coarse network enclosing islands or nests of tumor cells which frequently extended

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[3] Transplants made from lesions of this character were not so successful as those made from nodules composed of granular cells.

into the liver parenchyma beyond. Finally, some of the nodules showed concentric zones of tumor and connective tissue growth. The condition usually observed was that of a central mass of tumor cells surrounded by a more or less distinct zone of connective tissue followed by a second zone of tumor cells, then a zone of connective tissue, narrower than the first, and finally an outer zone of tumor growth diffusely invading the liver parenchyma. This condition is obviously suggestive of periods of increased activity on the part of the tumor cells or of decline in the resistance of the host and is comparable to the periodic growth frequently observed clinically in both man and animals.

The effect of the growth upon the liver itself was of two kinds. Apart from such effects as distortion of architecture and replacement of liver tissue by tumor growth, there was an advanced degeneration of liver cells associated with a moderate degree of hemosiderosis and necrosis of occasional cells or cell columns. The capillaries were moderately dilated but not congested. These conditions existed in parts of the liver which were not involved by the tumor growth.

A comparison of the growth in the liver with that in the scrotum or the inguinal lymph nodes is sufficient to indicate that the several groups of lesions were derived from a common parent cell. From the description of the growth in the liver and the reaction of the surrounding tissues, it is also apparent that differences in the structural characteristics of the lesions may be referred to such factors as age and rate of growth on the one hand, and on the other, to variations in the resistance encountered due to variations in functional capacity of the host.

*Lungs.*—Grossly, the lungs presented a normal appearance, except for the presence of numerous small opalescent or grayish white nodules distributed chiefly over the dorsal surfaces and through the substance of the two lower lobes. Microscopically, there were lesions of three types. The lymphatics and smaller blood vessels contained numerous collections of tumor cells with a granular, basophilic cytoplasm which formed lesions such as those in Fig. 22. In addition, there were larger nodules of growth composed of a mixture of granular and of vacuolated cells, while a few nodules were made up almost entirely of cells with a clear staining, vacuolated cytoplasm. Many of these lesions exhibited a peribronchial or perivascular arrangement. There was no reaction about the smaller pulmonary metastases and only a slight cellular infiltration in the alveolar walls surrounding the larger lesions.

*Kidneys.*—The kidneys presented an almost normal appearance. A single opalescent nodule measuring approximately 2 mm. in diameter was found in the inner portion of the cortex of one kidney. Microscopically, however, numerous collections of actively growing tumor cells were found in the capillaries of the glomerular tufts (Fig. 23), and in some instances, completely filled the glomeruli. A few lesions of similar character were present in the intertubular spaces of the cortex.

It is of interest to note that although there was no considerable tumor growth in the kidneys, there were well marked degenerative changes in both the tubular epithelium and the glomeruli, the latter condition manifesting itself as a hyaline thickening of the capillary walls (Fig. 23).

*Spleen.*—The spleen was a pale yellowish brown color, rather small, but distinctly swollen and contained five prominent nodules together with a number of smaller ones, which presented the same gross and microscopic appearance as those in the liver. In fact, the splenic pulp was largely replaced by tumor growth. The sinuses were filled with tongue-like projections and columns of tumor cells, while the Malpighian bodies were reduced to small proportions, being compressed between masses of tumor growth.

The walls of the arterioles showed a marked thickening; the capsule and trabeculæ were moderately thickened, while the sinuses were distended and rigid and small deposits of yellowish brown pigment were present in all parts of the organ.

*Bones and Marrow.*—At autopsy, the bones of the legs were rather fragile, while the ribs, the sternum, and the bones of the skull were decidedly softened. The medullary cavities of the femur, tibiofibula, humerus, ulna, and radius of both sides were widened and contained masses of tumor growth which in some instances almost filled the cavity. In other places, the growth appeared as small, clearly defined nodules. The bodies of the lower cervical and lumbar vertebræ were also involved. In the lumbar region, the growth projected into the neural canal and pressed upon the spinal cord. Other bones showed no macroscopic evidence of tumor growth and a complete histological examination was not made. Metastases were found, however, in the bones of the pelvis and were doubtless present elsewhere.

The bone metastases were extremely soft and flaky and were composed almost entirely of cells of the vacuolated type with little or no stroma and a very scant blood supply.

Microscopically, the bones showed a distinct widening of medullary spaces with a corresponding reduction in the thickness of the bone. The marrow was in a state of unusually active hyperplasia which was peculiar in that there were very few megacaryocytes and those present showed pycnotic and irregular nuclei with a scant and extremely ragged cytoplasm. Other cells, including both the myelocytic and erythroblastic series, were greatly increased, as indicated in Fig. 24 (middle of femur).

*Lymph Nodes.*—With the exception of the mesenteric and retroperitoneal groups, the lymph nodes were small, pale, and opaque and intensely indurated. The mesenteric nodes were also small but were deeply pigmented, while those extending from the pelvic region to the celiac axis were slightly swollen, translucent, and moist. Several of these nodes contained soft, opalescent nodules of tumor growth and microscopic examination revealed the presence of many others.

The structure of the metastases in the retroperitoneal lymph nodes differed somewhat from that in the inguinal nodes. There were many focal accumulations of tumor cells lying free in the peripheral and medullary sinuses or imbedded in the substance of the follicles (Fig. 25) which were composed of granular cells with a basophilic cytoplasm and showed numerous mitotic figures. The larger metastases, however, showed an extreme degree of colloid degeneration, and the growth

spread diffusely through all parts of the node with apparently no opposition. The irregularity and indefiniteness of cell outlines, the presence of giant cells not seen in other metastases, and the formation of a peculiar protoplasmic network which characterized the growth in these nodes are illustrated in Fig. 26. Another feature of these lesions was the absence of any stroma except that supplied by the preexisting framework of the node and the presence of few if any newly formed blood vessels.   Mitotic figures were fairly numerous but less frequent than in the smaller metastases.

There is some uncertainty as to the origin of the cells composing these lesions. As will be seen later, there was a tumor-like growth in the left testicle of this animal which was composed of very large cells some of which were not unlike the giant cell illustrated in Fig. 26. Hence, the occurrence of cells of this type in this particular location and their absence from other metastases raises the question as to whether these cells were derived from the scrotal or the testicular growth.

Apart from the presence of tumor metastases in isolated groups of nodes, there were decided alterations in all the lymphoid tissues. These were of two kinds. In the first place, there were a more or less complete disappearance of germinal centers and of characteristic germinal cells, or even follicular outlines in many nodes, and a reduction in the number, size, and clearness of definition of the centers in others. Some of these features are illustrated in Fig. 27. At the same time, many nodes showed irregular and, in some instances, rather conspicuous collections of epithelioid (endothelial?) cells throughout the cortex. The sinuses were usually dilated but contained comparatively few cells.

Offsetting these changes, there appeared to be a compensatory increase in the number of nodes, indicated by the frequency with which minute nodes were encountered in sections from various localities; some of these showed a preservation or even a hyperplasia of the germinal centers.

The superficial lymph nodes, as distinguished from those of the abdominal and thoracic regions, showed a distinct fibrous thickening of the capsule and stroma with a thickening of the vessel walls and occasionally a lymphocytic infiltration.

The alterations described in this group of tissues were doubtless due in part to the syphilitic infection but may have been influenced to some extent by the presence of the tumor, since similar changes have been noted in tumor-bearing animals in which no syphilitic infection could be demonstrated.

### Pathological Conditions in Organs Not Involved by the Tumor Growth.

While a great deal of valuable information can be acquired from a study of primary and secondary tumor growths and the reactions of surrounding tissues, it is doubtless recognized that fully as much may be gained from a careful survey of other conditions associated with the origin and progress of such a growth. The animal which formed the basis of this investigation presented a number of abnormali-

ties some of which appear to be significant with reference to the two major diseases—syphilis and tumor—and while at present we do not intend to discuss this phase of the subject in detail, it is necessary that the conditions referred to be described for the sake of completeness as well as for future reference.

Clinically, a pronounced scleroderma antedated the recognition of the growth in the scrotum, and post mortem, a number of other conditions were found; these included degenerative changes in the cardiovascular system, chronic inflammatory and degenerative processes associated with atypical epithelial proliferations in the tongue and buccal mucosa, esophagus, bronchi, and left testicle, fibrotic changes in the skin and subcutaneous tissues, and alterations in the endocrine glands.

*Cardiovascular System.*—The heart was normal, except for occasional foci of chronic myocarditis, the significance of which is uncertain, and a moderate degree of myocardial degeneration. The arterial system, on the other hand, was markedly affected. Many of the smaller arteries were diffusely thickened, while the thoracic aorta contained a series of small saccular dilatations. In these areas, the thickness of the vessel wall was diminished. Viewed from the inner side, there were numerous opaque white or yellowish white lines and dots distributed chiefly over the dorsal surface. There were no nodular thickenings or areas of calcification, however, such as are commonly seen in the aorta of the rabbit.

Histological examination showed the presence of extensive degenerative changes in the media of the aorta and many of the smaller vessels. The appearance presented was that shown in Fig. 28, taken from an artery in the region of the celiac axis. There was no inflammatory reaction associated with these lesions. The process began with a swelling and disintegration of the muscle and connective tissue elements, the elastic fibers showing less alteration. During the earlier stages, cells and intercellular substance gave a distinct basophilic staining reaction and the appearance suggested a mucoid degeneration, but as necrosis developed, the reaction changed, the entire area staining deeply with eosin.

The affected parts of the vessel wall were at first thickened (Fig. 28), but with extension of the necrosis, there was a tendency to a reduction in thickness as may be seen in Fig. 29, taken from the outer edge of an aneurysmal dilatation in the aorta.

A third type of vascular lesion, consisting of a fibrous thickening of the outer coats together with some narrowing of the lumen, has already been referred to in connection with the lymph nodes (see Fig. 27). The nutrient vessels in all parts of the body exhibited changes of this type but they were most pronounced in such tissues as the lymph nodes and the skin.

*Skin and Subcutaneous Tissues.*—At autopsy, the skin over the ventral surface of the body cut with great resistance. In some places, it was freely movable but was everywhere firmly united to the deeper tissues by coarse and extremely tough strands of fibrous tissue. The subcutaneous tissues were unusually dry. The skin itself was diffusely thickened, the thickening being most marked over the trunk and about the head. Measurements made with calipers after removal cf the hair showed a thickness of from 2 to 6 mm. The greatest thickness was in the region of the right shoulder and involved an area approximately 10 cm. square. The measurement at the center was 6 mm., which diminished to 4 mm. at the periphery. The skin over other parts of the thorax varied between 3 and 4 mm. in thickness, along the midline of the back, 2 to 3 mm., and 2 mm. over the abdominal wall. The measurements for these regions are nearly double those of a normal animal of the same type and age.

In addition to the diffuse thickening, there were a number of small shotty nodules in the deeper layers of the skin, chiefly on the back. These varied from minute points to nodules measuring 2 to 3 mm. in diameter.

The thickest portions of the skin were of a leather-like texture, the corium being composed of coarse bundles of pale straw-colored tissue of a fibrous nature. On cross-section, the circumscribed nodules closely resembled the cut end of a nerve.

Thus far, it has been impossible to obtain satisfactory sections of these lesions for microscopic examination, except from the outer edges. Sections from both the diffuse and circumscribed lesions showed obvious abnormalities in the development and distribution of hair follicles as well as the structure of the corium. These conditions may be illustrated by Figs. 30 and 31, which were taken from the outer edge of a small nodule in the skin of the right flank. The structure of this nodule and its relations to other parts of the skin are shown in Fig. 30. It will be seen that the nodule is made up of a collection of large and irregular hair follicles surrounded on all sides by a thick and compact zone of connective tissue forming an elliptical mass which is not clearly demarcated at its periphery. The matrix of the nodule was composed of extremely coarse and angular bundles of hyaline connective tissue as shown in Fig. 31, which may be compared with other figures taken at the same magnification, such as Figs. 11 and 12. There were very few blood or lymph vessels in this tissue, and the walls of all vessels were thickened with a consequent narrowing of the lumen. Cellular elements were also scarce and were confined to a narrow zone immediately surrounding the vessels. The elastic tissues have not as yet been studied on account of technical difficulties.

The hair follicles were abnormal in size, location, arrangement, and structure. Individual follicles varied from minute or atrophic structures on the one hand to irregular giant follicles with radially arranged cells and cortical differentiation on the other, or to follicles of a large or small size composed entirely of undifferentiated germinal epithelium (bulb cells) showing numerous mitotic figures. Several of these structures are shown as dark bodies at the center and toward the extreme right of Fig. 30.

Individual follicles as well as the nests were encircled by connective tissue which was more nearly normal in appearance than that of the body of the nodule, but even here there was some tendency to degeneration and small foci of lymphocytic infiltration were occasionally seen.

The follicles in the papillomatous patches on the lips, which were referred to in the clinical history, presented a similar series of changes. In this instance, however, the tendency was almost entirely toward the production of giant follicles with cortical differentiation analogous to those of the pilot hairs or bristles about the face.

It will be seen that the peculiarities in the growth and arrangement of the hair follicles in the lesions described were not unlike those which existed in the skin surrounding the first and second tumor recurrences or even about the primary tumor itself, and they suggest the existence of a rather widespread abnormality affecting the growth and differentiation of cells forming this group of structures. This possibility is strengthened by the occurrence of an analogous group of changes in the epithelium of the tongue and buccal mucosa, the esophagus, and the testicle, which have not yet been described. However, it should be borne in mind that all these conditions were associated with equally marked alterations in the supporting connective tissue.

*Tongue, Buccal Mucosa, and Esophagus.*—The lesions present in these organs may be considered together since they represent processes of much the same character and significance. The sides and dorsum of the tongue (Fig. 32, *a*, to be compared with *b* which is normal) showed a series of irregular depressed areas which were smooth and rather translucent in comparison with intervening portions of the mucous membrane which appeared to be thickened and of an opaque white color, especially toward the base and sides of the tongue. Similar markings were found on the mucous membranes of the cheeks.

Microscopically, the depressed areas showed degeneration, necrosis, and exfoliation of surface epithelium associated with a chronic inflammatory reaction in the tissues beneath (Figs. 33 and 34). These changes resulted in destruction of papillæ with consequent smoothing of the surface and a reduction in the thickness of the epithelial covering. There was, however, an active proliferation of epithelial cells in all parts of the mucous membrane, and in the more opaque areas, there was a marked increase in thickness instead of a reduction, with irregular downgrowth of epithelium, as shown in Fig. 35.

The buccal mucosa and the mucous membrane of the upper part of the esophagus contained lesions analogous to those of the tongue. An example of the esophageal condition is given in Fig. 36.

The lesions included in this group are of double interest. The condition of the tongue is analogous in some respects to smooth atrophy and in others to leucoplakia of human syphilis, and is the only instance on record in which such lesions have been observed in an

animal infected with *Treponema pallidum*. The second feature of interest is the existence of chronic inflammatory processes associated with atypical epithelial proliferations in much the same manner as at the point of origin of the original tumor.

*Testicles.*—The testicles again presented a combination of two conditions. Both testicles were extremely small and on microscopic examination showed atrophy of the seminiferous tubules, slight interstitial fibrosis with considerable thickening of the tunics together with foci of round cell infiltration, chiefly about the vessels of the tunics. In the outer side of the left testicle, there was a mass the size of a small bean which was separated from the remainder of the organ by a thin wall of fibrous connective tissue. It was composed of imperfectly outlined tubular or alveolar structures surrounded by a well developed stroma of fibroblastic cells (Fig. 37). The alveoli were much smaller than the seminiferous tubules and contained large, irregularly shaped cells with vesicular nuclei and a granular cytoplasm which varied in staining reaction from a pale pink to a purplish blue color. The cells were larger than those of the seminiferous tubules but bore no resemblance to the cells of the scrotal tumor. In some places, they preserved the tubular arrangement, while in others, they formed compact and rather irregular alveoli, and occasionally one or more cells appeared to lie free in clefts of the stroma as at the point indicated by the arrow in Fig. 37. The cells were everywhere in a state of active mitotic division but nowhere was there a suggestion of spermatogenesis.

This lesion, if not an independent neoplastic growth, is at least a striking example of a tendency to atypical cell proliferation. The list of tissues showing increased or atypical proliferation of epithelial cells with inflammatory or degenerative changes in the supporting connective tissue, might be farther extended, but the instances cited are sufficient to emphasize this feature of the pathological status of the animal under consideration.

*Endocrine Glands.*—The thyroid and suprarenals showed decided abnormalities, while changes of a less definite character were present in the thymus and pituitary body.

The thymic mass was of approximately normal size but was composed chiefly of fat with very little thymic tissue.

The thyroid was quite small and of a pale grayish pink color. Microscopically, the outstanding features were irregularity in the size and shape of acini, relatively scant, pale staining, and vacuolated colloid, and an increase in the interstitial tissue. Some of the acini presented an essentially normal appearance, others were small and contained little or no colloid, while the acini of a third group were large and irregularly shaped and the cells lining these acini were large and cuboidal or columnar with irregular nuclei and a decidedly granular cytoplasm; they were frequently several layers deep or formed so called epithelial buds. The colloid in this group of acini stained a pale pink and was extremely vacuolated or even granular in places.

The stroma of the gland was increased and there were a few small areas of lymphocytic infiltration. Some of the blood vessels were slightly thickened, but on the whole they showed no decided abnormality.

The suprarenals, as in the case of the thyroid, were extremely small for an animal of this age and were of a deep orange-yellow color. The medullary area was relatively large, while the cortical zone was comparatively narrow. Microscopically, the cortical cells showed extensive degeneration and necrosis with numerous mitotic figures in the zona glomerulosa. The changes in the pituitary were less definite. The anterior lobes were unusually prominent, and, microscopically, the cells composing this part of the gland were practically all of one type, their protoplasm containing fine dust-like granules which stained a pale lilac-pink. Other parts of the gland presented an essentially normal appearance.

## DISCUSSION.

It would be impossible to undertake a comprehensive analysis of the conditions described in the preceding pages without the introduction of experimental data, which is not advisable at this time. Hence, discussion will be limited to such subjects as can be dealt with upon the basis of material contained in this paper.

It may appear that the conditions which have been described form a rather heterogeneous collection of pathological processes which for the most part bear no relation to one another, but a more careful analysis will show that they are separable into three fairly distinct but closely related groups of phenomena. The first group includes a growth which originated in the scar of a chancre following a mild but persistent syphilitic infection; the second consists of a series of chronic inflammatory conditions associated with active and atypical proliferation of epithelial cells; while the third group of conditions includes a variety of retrogressive processes of widespread distribution, some of which antedated the appearance of the growth in the scrotum while others were of later development and in some instances appear to have been terminal events in the course of the disease. It is apparent that in making this classification there is an unavoidable overlapping of the several groups in that conditions assigned to one group also enter as a part of the processes included in the others.

Clinically, the growth in the scrotum was indistinguishable from a cutaneous syphilide, but its history and the results of pathological examinations have removed all doubt as to its neoplastic nature.

There may be some question, however, as to the proper classification of the tumor. The metastatic lesions in particular present a picture which is usually associated with sarcomata or at least with tumors of mesodermal origin, but a careful examination of a large series of sections of the primary and recurrent growths with surrounding skin areas has led to the conclusion that the tumor is of epithelial origin. It is obvious, however, that the growth does not conform to either the squamous or basal cell type of epithelioma commonly seen in man, although it might be included in the latter group.

The nearest analogy in cell type among normal epidermal structures is found in the cells composing the hair follicle, especially those of the bulb and outer root sheath, and it appears that the cells forming the tumor have followed this line of differentiation. The peculiarities of the tumor might be accounted for, therefore, upon the basis of developmental tendencies of epithelial cells. Thus, in man, the dominant tendency is apparently in the direction of the development of a protective covering of epithelium, the production of hair being subsidiary, whereas in the rabbit, conditions are reversed; the epidermis in general is less highly developed and the protective function is subserved to a greater extent by the growth of a heavy covering of hair. In this animal, as has been pointed out, there were obvious developmental abnormalities of the hair follicles in many parts of the skin which may in itself be of some significance.

No explanation of the origin of the tumor will be offered at this time, but there are certain facts bearing upon the subjects of etiology and malignancy which may be reviewed briefly in order to make clear the nature of the problem presented.

The history of the growth stamps it as a typical instance of the development of a malignant tumor upon the basis of a chronic inflammatory lesion in which a syphilitic infection may have played the part of a predisposing or of an exciting factor. It is apparent that the growth began as a process of atypical epithelial proliferation such as is common to chronic inflammatory lesions of the skin and probably differed in no essential respect from the proliferative changes present in neighboring parts of the skin or from those which subsequently developed in the mucous membranes of the mouth and esophagus.

The time at which the transition to an independent neoplastic growth occurred is not clearly indicated. From the history and from the composition of the lesion first removed from the scrotum, it is certain, however, that this change took place gradually and that there were periods of ineffectual activity before the growth finally became established and assumed the characteristics of a malignant tumor. Even then the progress of the growth was not uninterrupted, but was marked by periods of quiescence or temporary regression such as are frequently observed in other tumors.

It should be borne in mind, however, that these features of tumor growth are not peculiar to tumors but that they represent changes of a type well recognized in lesions or diseases which pursue a relapsing course, and are identical in character with the course of events ordinarily observed in syphilitic infections. Moreover, this feature of the growth was no more than a continuation of the phenomena of relapse which had been taking place in the lesion from which the tumor originated, and in other lesions in this animal. It is apparent, therefore, that the conditions responsible for these changes were not necessarily associated with the tumor itself and that they were not of a purely local character.

A second feature of this case which is deserving of consideration concerns the occurrence of fibroid lesions in the dermis which were noted several months prior to the recognition of the growth in the scrotum. At first, there was only a moderate diffuse thickening of the skin, but eventually, the condition assumed marked proportions with nodular and diffuse lesions. The association of three distinct conditions such as scleroderma, leucoplakia buccalis, and a malignant neoplasm in one individual is most unusual, but the tissue changes in these processes do not differ materially from those frequently observed in cases of malignant disease. As has been pointed out, the lesions of the buccal mucosa and esophagus were of much the same order as that from which the tumor arose, and the fibroid changes in the skin will be recognized as a process of essentially the same character as that seen in the connective tissue of so called scirrhous tumors. In fact, the focal lesions were composed of atypical epithelial growths in the form of hair follicles with a matrix of hypertrophied and hyaline connective tissue, and similar, although less marked changes were

present in the connective tissue surrounding the primary growth and many of the earlier metastatic lesions.

The development of the diffuse fibrous thickening in the skin was undoubtedly influenced by frequent handling over a period of years, but this merely served to accentuate the condition and thus brought out a latent weakness in this group of tissues which otherwise might have escaped notice. The change was progressive, however, and increased after the development of the tumor. In like manner, the growth of connective tissue, which had been feeble at all times, became less and less active, until at the time of death there was little or no connective tissue reaction about metastatic lesions and there was complete failure of union in the operative wounds. Finally, chronic fibrous thickening and acute degenerative changes were found in the walls of the blood vessels, which suggests a course of events not unlike those seen in the skin and in the connective tissue about tumor growths.

Retrogressive changes were not confined to this group of tissues, however, but were present in the parenchymatous organs as well and were especially evident in the lymphoid tissues, the thymus, the thyroid, and the suprarenals. It is thus apparent that there was widespread deterioration of organs and of tissues, much of which antedated the development of the tumor; hence, the tumor did not arise in an organism which was otherwise normal but in one with obvious organic impairment which increased with the progress of the growth.

Moreover, neoplastic changes in the epithelium were not confined to a single focus or even to a small area of the skin but spread in a gradually widening circle and with increasing rapidity, until a considerable part of the skin over the lower abdominal region exhibited changes which differed from those of the original growth chiefly in that they were no longer focal but diffuse in character. At the same time, atypical epithelial proliferations were taking place in other parts of the body and at one point (the left testicle) a growth developed which might be regarded as a second epithelial tumor.

This statement of the circumstances which attended the development and growth of the tumor under consideration will serve to indicate the complexity of the problems of etiology and malignancy

and the necessity for taking into account conditions other than those of a purely local character. In fact, there is every indication that the factor of foremost importance in determining the course of events in this instance was constitutional rather than local. Further than this we shall not go until the results of our experimental work have been reported.

### SUMMARY AND CONCLUSIONS.

A malignant tumor which developed at the site of a primary syphilitic lesion in the scrotum of a rabbit, about 4 years after inoculation, and eventually led to the death of the animal, has formed the basis for an extensive series of investigations beginning with the study of the spontaneous tumor. The present report contains the clinical history of the animal from the time of inoculation to its death and the results of pathological examinations.

The most important events recorded may be summarized as follows:

1. A mild but persistent syphilitic infection with an occasional relapse and the eventual development of chronic inflammatory lesions in the skin associated with atypical epithelial proliferations.

2. The occurrence, at about the same time, of extensive degenerative changes in the dermis accompanied by an atypical growth of hair follicles, with the production of diffuse and nodular areas of thickening and induration (scleroderma).

3. The development of a growth in the skin of the left scrotum which recurred after removal and spread diffusely over adjacent parts of the skin, and metastasized to the regional lymph nodes and to internal organs.

4. The transplantation of the growth to other rabbits by intratesticular inoculation and the successful propagation of the growth over a period of nearly 2 years (twenty generations).

5. The development of a cachexia and of pressure phenomena from metastases involving the cervical and lumbar regions of the spinal column which eventually led to the death of the animal.

6. The discovery, post mortem, of an extensive leucoplakia of the tongue and buccal mucosa, chronic inflammatory lesions in the esophagus with atypical epithelial proliferation, and a nodular growth in the left testicle differing in character from that in the scrotum.

7. The presence of extensive degenerative changes in the vascular system, degeneration of the parenchymatous organs, atrophy of the thymus and lymphoid system, with chronic lymphadenitis, atrophy, degeneration, and necrosis of the suprarenals, and atrophy and hyperplasia of the thyroid with chronic thyroiditis.

The conclusion was reached that the growth in the scrotum represented a neoplasm of epithelial origin which was composed of cells allied to those found in the bulb and root sheath of the hair. No explanation was offered for the origin or unusual malignancy displayed by the tumor pending the introduction of experimental data. It was stated, however, that the evidence presented was sufficient to warrant the assumption that the factor of foremost importance in the development of the tumor was constitutional rather than local.

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### EXPLANATION OF PLATES.

The illustrations are reproductions of photographs which have not been retouched. Gross objects are represented at their natural size. The photomicrographs were taken from sections stained with hematoxylin and eosin or with methylene blue and eosin (Figs. 16 and 17).

### PLATE 31.

FIG. 1. Chronic inflammatory reaction in the left scrotum 3 years and 7 months after inoculation.

FIG. 2. Primary growth in the left scrotum 4 years and 4 months after inoculation.

FIG. 3. Recurrence of the tumor growth in the left scrotum with metastases in the inguinal nodes 3 months after excision of the primary growth. The scar from this operation is seen below the recurrent growth.

FIG. 4. Cross-section of the primary tumor with part of the surrounding skin area. The points marked on this figure refer in some instances to exact areas described or illustrated in succeeding photographs or to general localities in instances in which the photographs were taken from other sections of the same growth. See the text for descriptions.   × 12.

Fig. 5. Epithelioid type of growth from the oldest of the three nodules composing the primary tumor mass (a, Fig. 4). × 450.

## PLATE 32.

Fig. 6. Primary growth. Changes in the epidermis and papillary layer of the skin in the region of b, Fig. 4. × 200.

Fig. 7. Primary growth. Epidermis and papillary layer of the skin showing intracystic growths of epithelium. The same general area as Fig. 6. × 200.

Fig. 8. Primary growth. Cells from the interior of nodule B (c, Fig. 4). × 900.

Fig. 9. Primary growth. Arrangement of cells and structure of growth in the same area as Fig. 8. × 450.

Fig. 10. Part of the primary growth from area e, Fig. 4. This section shows the origin of the tumor cells from the epidermis with a gradual transition in the appearance of the cells as the growth extends downward and becomes continuous with nodule B. × 200.

Fig. 11. Primary growth. Tumor cell emboli in the lymphatics of the skin outside the area of tumor growth (f, Fig. 4). × 200.

## PLATE 33.

Fig. 12. Part of an accessory growth in the skin above the primary nodule (g, Fig. 4). Note the general hyperplasia of the epithelium, the arborization of the epithelial process, the transition to basal cells, the perilymphatic arrangement of the basal cells, the sharp delimitation of the growth, and the absence of keratinization and of cellular reaction. × 200.

Fig. 13. Part of an accessory growth from the same area as Fig. 12. The section was taken from the periphery of an atypical hair follicle and the growth simulates this structure. × 200.

Fig. 14. Atypical epithelial proliferation with chronic inflammatory reaction in the skin at the outer edge of the tissue removed with the primary growth. This is of interest in connection with the recurrent growth. × 200.

## PLATE 34.

Fig. 15. Diffuse epithelial growth from the skin at the outer edges of the first cutaneous recurrence. Note the similarity between the growth of epithelium in this section and that in Figs. 6, 10, and 12. × 200.

Fig. 16. Atypical growth of hair follicles in the skin about the first cutaneous recurrence. × 200.

Fig. 17. Metastasis, left inguinal node. The section shows the general character and distribution of cells forming these lesions, areas of necrosis, fibrous thickening of the capsule and vessels, and invasion of tumor cells. × 200.

PLATE 35.

FIG. 18. Convex surface and cross-sections of the liver showing the appearance of the metastatic nodules.

FIG. 19. Liver. Part of an old metastatic nodule surrounded by a thick wall of dense fibrous tissue which is being invaded by the tumor cells. Note the type of tumor cell composing this growth as contrasted with those in Figs. 20 and 21. × 450.

FIG. 20. Liver. A more recent lesion showing colloid or mucoid degeneration of tumor cells with slight reaction at the outer margins. × 450.

PLATE 36.

FIG. 21. Liver. A third type of growth which combines some of the features of the two preceding lesions but is more malignant in character than either of these. × 450.

FIG. 22. Lung. Emboli of tumor cells in pulmonary vessels. × 450.

FIG. 23. Kidney. Tumor cell emboli in glomerular capillaries. Note also the hyaline thickening of the capillary walls and the degeneration of the tubular epithelium. × 450.

PLATE 37.

FIG. 24. Femur. Hyperplasia of the bone marrow. × 450.

FIG. 25. Retroperitoneal lymph node from the celiac region. Mass of tumor cells in a peripheral sinus with beginning growth in the substance of the node. × 200.

FIG. 26. Retroperitoneal lymph node from the lower lumbar region. A diffuse tumor growth of the colloid type containing mononuclear giant cells which resemble those of the growth in the left testicle. Compare with the cells of the scrotal tumor in Fig. 8, taken at the same magnification, and with the testicular growth in Fig. 37, which is half this magnification. × 900.

FIG. 27. Superficial cervical lymph node. Note especially the absence of germinal centers and the thickening of the capsule, and the walls of vessels in the node and surrounding tissues, indicated by arrows. × 50.

PLATE 38.

FIG. 28. Artery from the region of the celiac axis showing degeneration and necrosis in the media. Note the increased thickness in these areas. × 50.

FIG. 29. Thoracic aorta. Longitudinal section at the outer edge of an area of dilatation. The thickness of the wall diminishes at this point and there is almost complete necrosis of the muscle and connective tissue elements. The elastic fibers are fairly well preserved. × 100.

FIG. 30. Section of the outer edge of an indurated cutaneous nodule from the ventral surface of the right flank. The drawn lines indicate the general outlines of the nodule which is composed of nests of atypical hair follicles surrounded by

coarse bundles of hyaline connective tissue, the individual fibers of which are greatly enlarged. × 12.

FIG. 31. Hypertrophy and hyaline degeneration of connective tissue from the center of Fig. 30. Compare with Figs. 11, 12, and 14 at the same magnification and note the absence of capillaries and connective tissue cells. × 200.

## PLATE 39.

FIG. 32, a and b. Upper surface of the tongue from specimens preserved in Kaiserling's solution. (a) Syphilitic-tumor rabbit. (b) Normal rabbit. The distortion in a is largely artificial and accidental. The irregular markings indicate the important surface abnormalities.

FIG. 33. Cross-section of tongue, a in Fig. 32, showing necrosis and erosion of the outer layers of epithelium in a translucent area. There is a moderate chronic inflammatory reaction in the mucosa with perivascular infiltration and slight hyperplasia of the epithelial cells. × 100.

FIG. 34. A more advanced stage of the same process that is shown in Fig. 33. Papillæ are entirely absent from this area and the epithelium is deeply eroded, while the inflammatory reaction and epithelial hyperplasia are increased. × 100.

FIG. 35. Tongue. Atypical hyperplasia of epithelium from one of the more opaque white areas toward the sides of the tongue (a, Fig. 32). A leucoplakia-like lesion. × 100.

## PLATE 40.

FIG. 36. Esophagus at the level of the larynx. Atypical hyperplasia of the epithelium with areas of degeneration and necrosis associated with a slight inflammatory reaction. There are numerous mitotic figures in the lower layers of the surface epithelium and in the gland-like structures of the deeper tissues. × 200.

FIG. 37. Section of the growth in the left testicle. The tubular structures composing this lesion were from one-quarter to one-half the size of the atrophied seminiferous tubules, while the cells averaged two to three times the size of those in the tubules. The outlines of the tubular structures are lost in places and some of the cells are free in the fibroblastic stroma. Compare these cells with those of the metastatic growth in Fig. 26. There are numerous mitotic figures in all parts of the section. × 450.
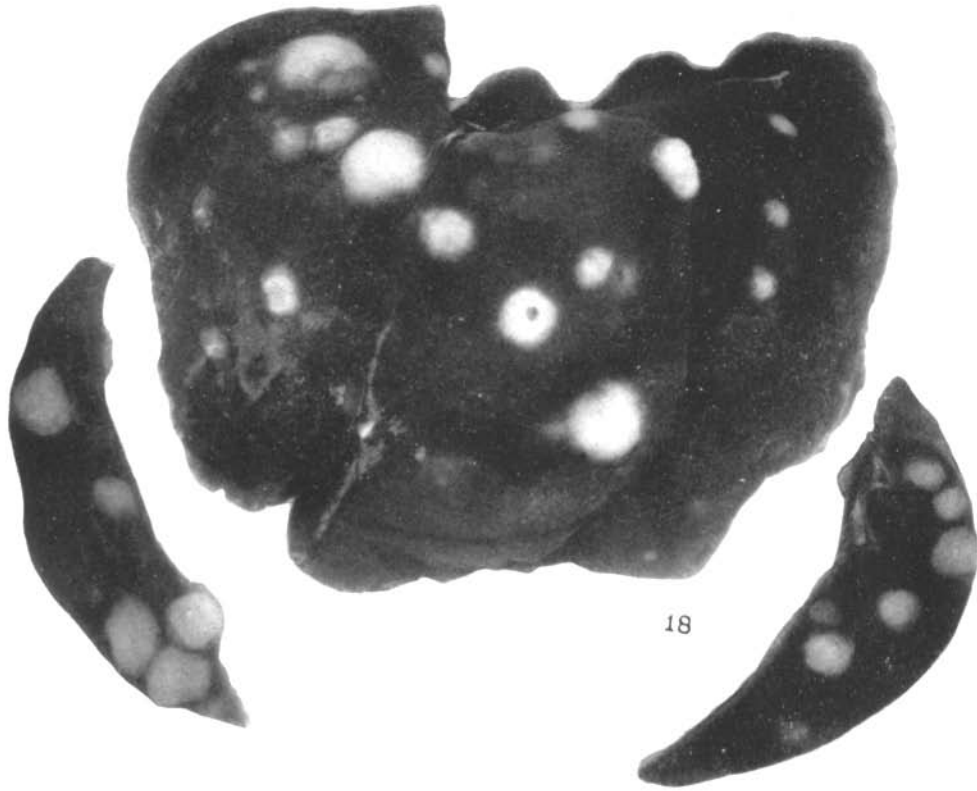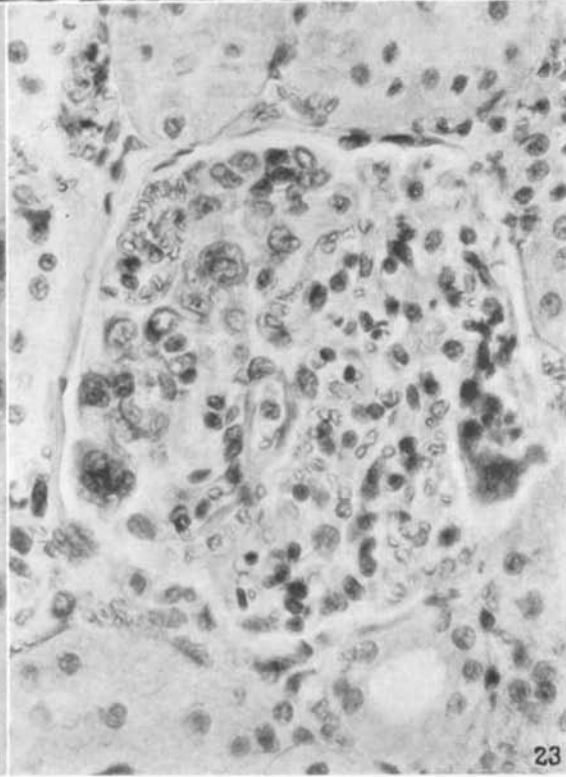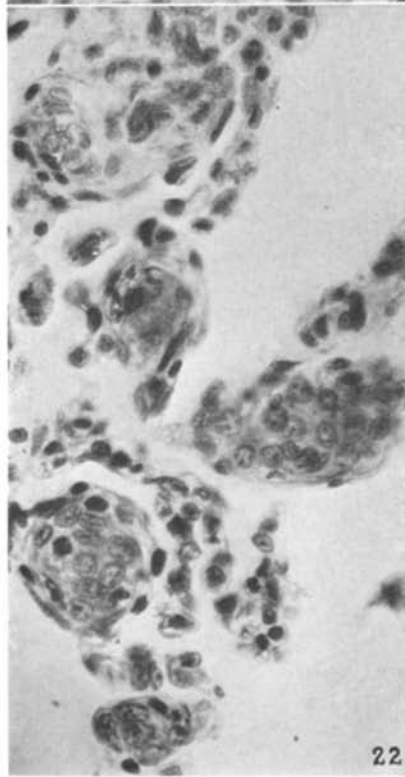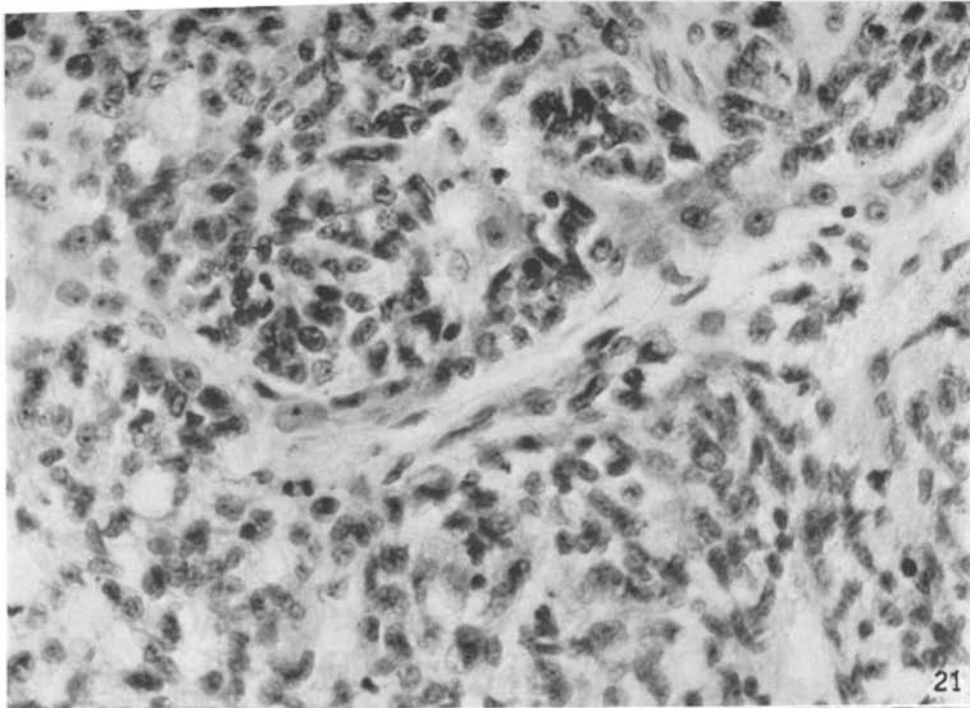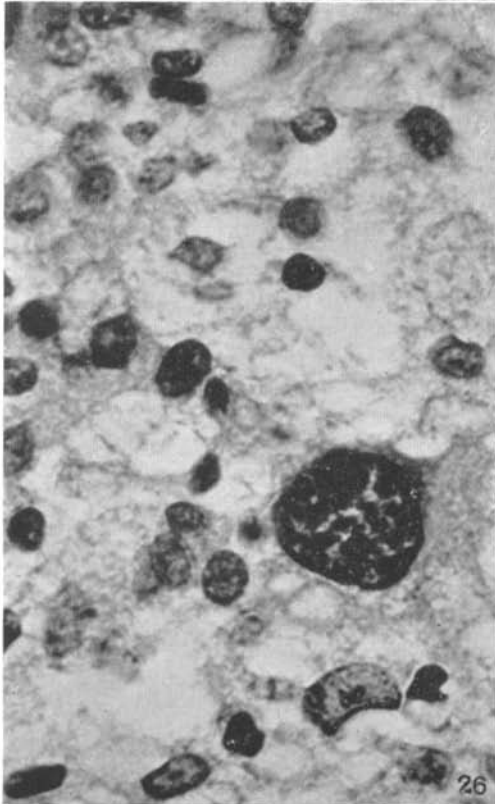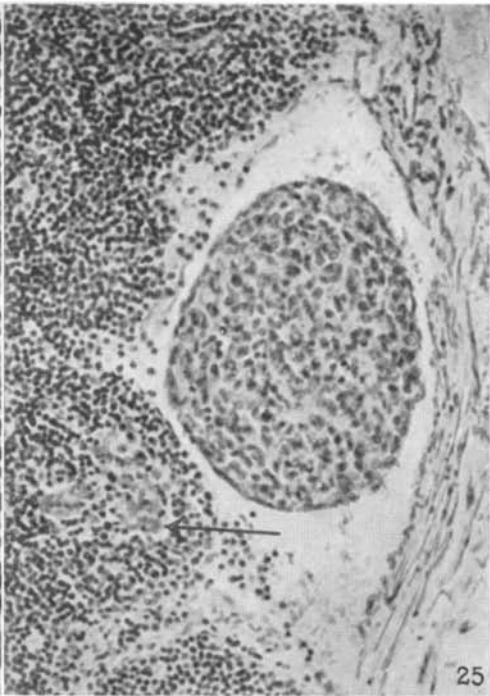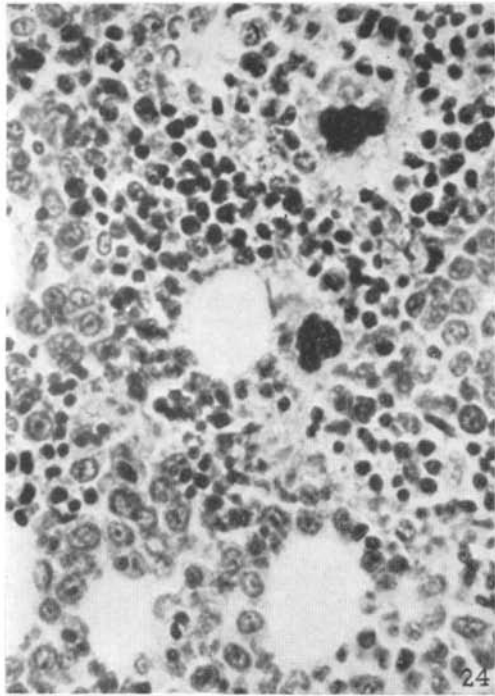
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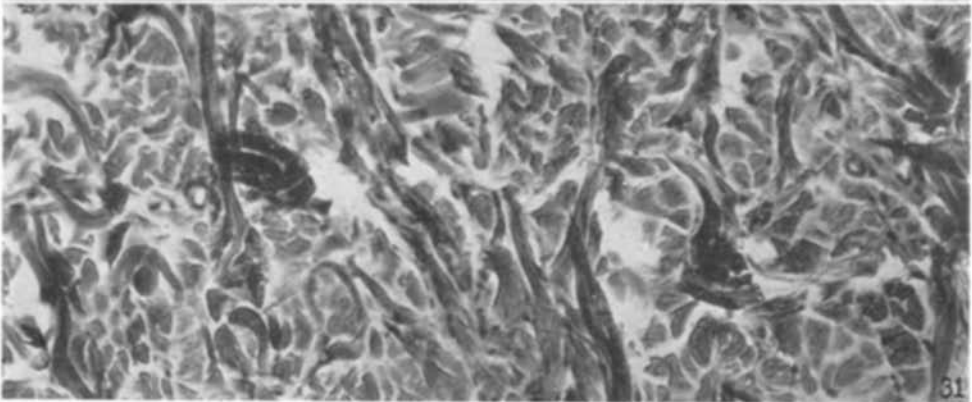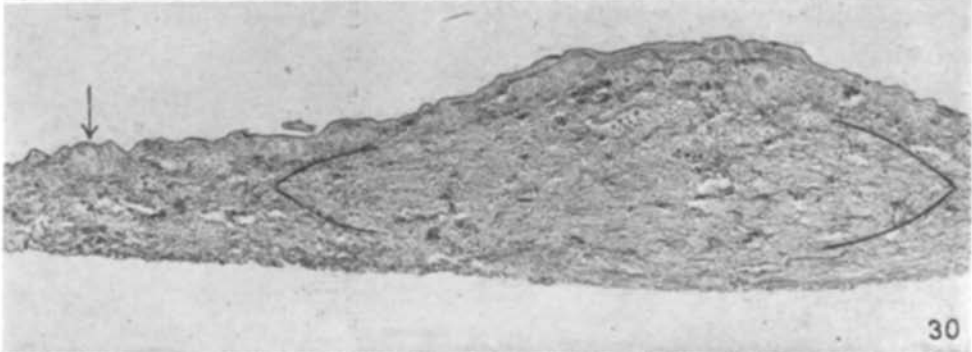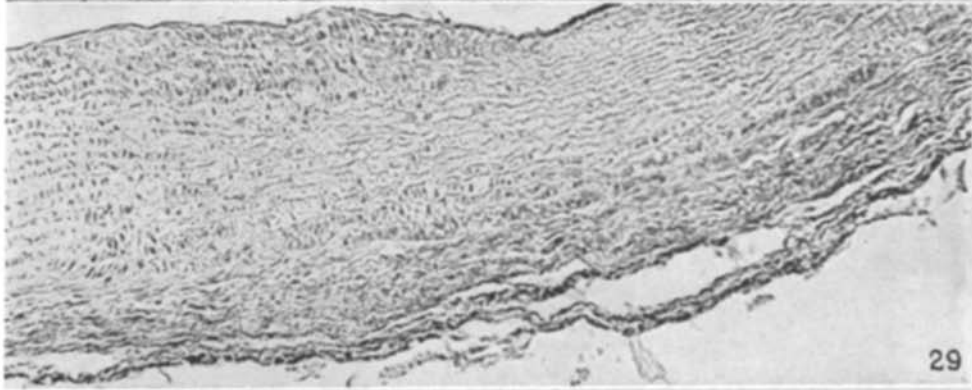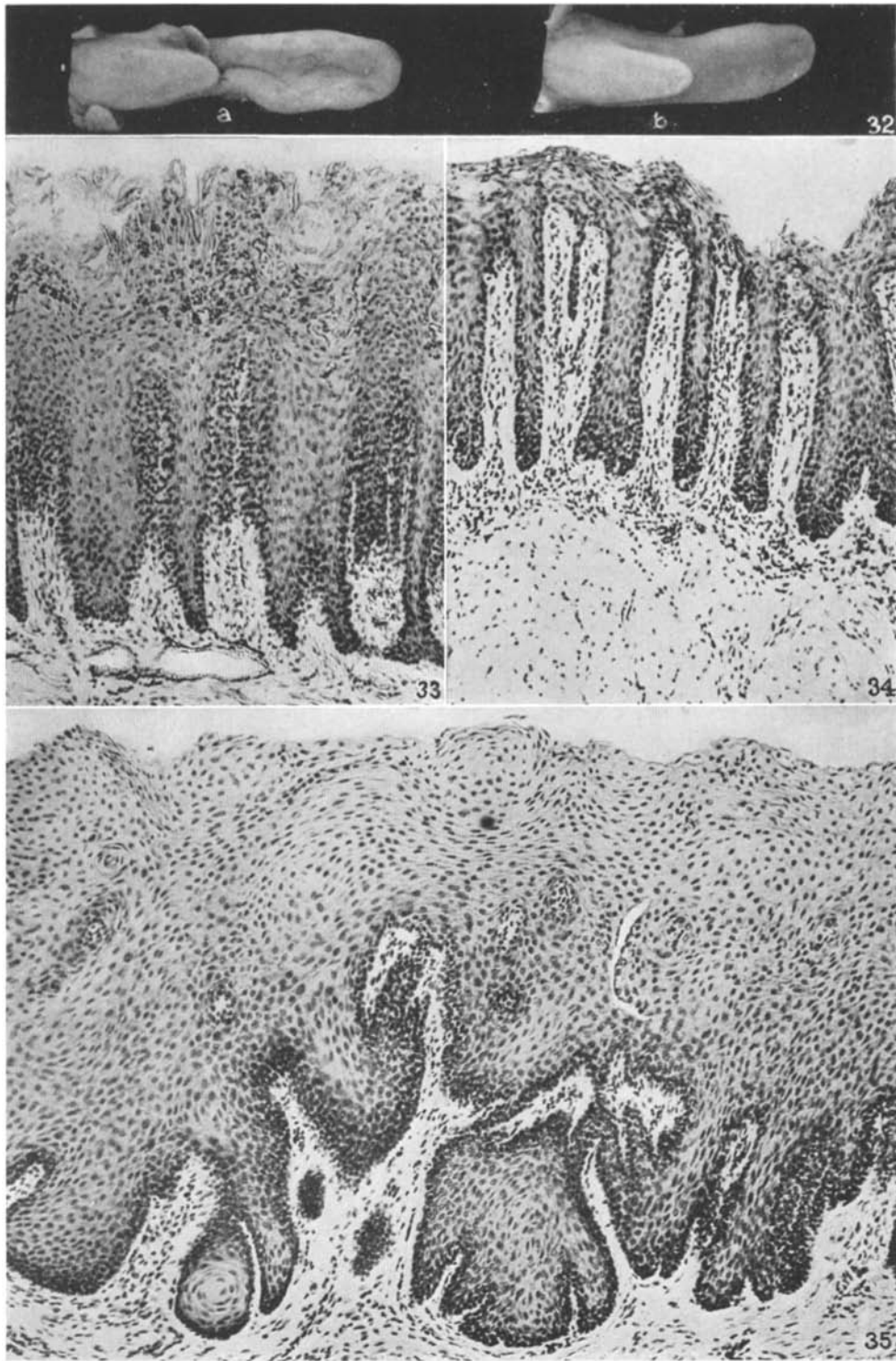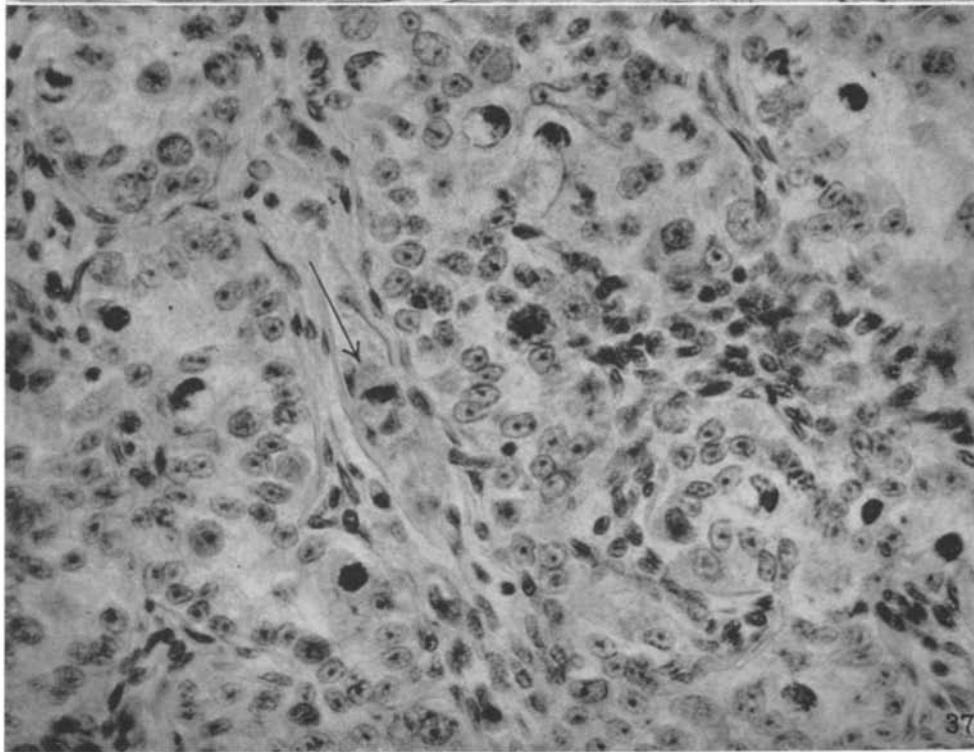
(Brown and Pearce: Malignant tumor of the rabbit.  I.)

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PLATE 38



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