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A STUDY OF THE SO-CALLED INFECTIOUS LYMPHOSARCOMA
OF DOGS. *

S. P. BEEBE AND JAMES EWING.

(*From the Huntington Fund for Cancer Research, and the Department of
Pathology, Cornell University Medical College.*)

For many years the attention of pathologists has been attracted by a venereal disease of dogs, characterized by transmission through coitus, by the presence of nodular tumors of the genitals, multiple secondary growths of the skin, metastases in the inguinal and abdominal lymph nodes, accompanied by cachexia, and often terminating fatally.

The most significant feature of the disease has been the marked infectiousness of the process, both by natural contagion and by experimental inoculation, but the important bearing of the malady on the general problem of tumors and the unusual facilities which it offers for the experimental study of a bulky tumor readily transmissible between comparatively large animals seem not to have been fully recognized until recently.

Clinical features. — The disease is evidently of general distribution, having been described in Russia, France, Germany, and England, and we have had no difficulty in securing numerous cases in New York City.†

Young or middle-aged adult dogs of both sexes are chiefly affected. Instances of the spontaneous transference of the

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disease *per coitum* are reported in detail by Smith and Washbourn, and Powell White, and are commonly observed by veterinary surgeons. In the female the disease begins with small nodular or papillary growths, single or multiple, of the vaginal mucosa, whence it extends by metastasis to the neighboring skin, pelvic, and abdominal lymph nodes, and occasionally to more distant organs. In the male the corona glandis is usually first involved by a nodular infiltration with secondary cutaneous tumors, sometimes involving the entire penile sheath. A catarrhal inflammation usually precedes the development or discovery of the tumor, but in many cases the growth appears without any inflammatory irritation. In both sexes isolated growths of the skin may occur in the groin, abdomen, back, neck, and hind or fore legs, as well as in the abdominal lymph nodes, mesentery, spleen, liver, kidney, and lung.

The superficial tumors may ulcerate or necrose, but the protected growths are usually found intact. The growths are usually well circumscribed, encapsulated, and free from any signs of inflammatory reaction, but in older cases there may be diffuse infiltration of the skin or mucous membrane which, becoming eroded or ulcerated, may be considerably inflamed and yield a serous or purulent discharge.

In the larger tumors considerable areas may necrose and be sloughed out. Spontaneous regression may occur through a process of fibrosis and atrophy, and permanent disappearance of the disease in this manner has been noted. Local treatment has considerable influence in checking the progress of the disease if instituted early, and Dr. Sherwood informs us that by repeated curettage and application of caustics the early stages of the genital lesions may usually be arrested.

Clinical conditions in the dog resembling infectious lymphosarcoma. — Sticker describes two infectious inflammatory diseases of dogs which resemble the infectious lymphosarcoma. (1) The pointed condyloma of the genitals arises after long continued irritation and produces small papillary excrescences which often reach a considerable size and may

produce large firm lobulated outgrowths. Histologically they consist of hypertrophic epithelium supported by new vascular connective tissue infiltrated with round cells. (2) An infectious follicular vaginitis or balanitis arises in the course of chronic purulent catarrh and produces multiple tumors of the size of a pea or bean, composed of enlarged lymph nodules which displace the epithelial lining outward.

We have encountered in Dr. Sherwood's clinic cases apparently belonging to the class of follicular vaginitis, but we do not feel certain that such cases can be satisfactorily distinguished from early lymphosarcoma. According to Sticker the true tumor is usually single and may be unaccompanied by catarrhal inflammation, and its cells are of large size, while follicular vaginitis gives multiple superficial tumors, is always marked by purulent discharge, and the lymphoid cells are smaller. Yet Smith and Washbourn state that a constant leucorrhea is the usual sign of the presence of this tumor, and the multiple tumors which we have examined in cases of vaginitis have shown much the same structure as the metastatic lymphosarcoma. We are inclined, therefore, to suspect that there is a close connection between Sticker's follicular vaginitis and the true lymphosarcoma which many authors have been studying.

Dr. Sherwood assures us that all cases of vaginitis in dogs with submucous nodular growths, if untreated, go on to develop the characteristic lesions of lymphosarcoma, and that the only other form of chronic vaginitis in dogs, as in the human subject, produces only small granular elevations of the epithelium, but never any definite tumors of the size of a pea or bean. We are, therefore, not ready to accept Sticker's description of an infectious inflammatory hyperplasia of the submucous lymph nodules as a separate disease from the lymphosarcoma of various authors.

(3) In one case, which we have recently observed with Dr. Sherwood, an old dog presented much more extensive lesions of the type of lymphosarcoma than any we have found described. The skin of the genitals and groin was thickly infiltrated with many flat masses over which the skin

was eroded, dry, and shiny. There were several large tumors and many smaller ones on the back, and hind and fore legs. The skin of the lips, face, and head was beset by a large number of nodules or flat plates covered by reddened shiny cuticle. The lymph nodes of the groin, pelvis, abdomen, and those about the jaws and neck were much enlarged by extensions of the tumor process. The spleen was nearly doubled in size by many small nodules which had the distribution of the Malpighian bodies. On section of this organ, the pulp was found to contain many islands of nucleated red blood cells interspersed with many typical myeloid giant cells. In the liver the portal canals were extensively infiltrated by masses of small round cells. The structure of all these tumors was that of small cell lymphosarcoma. On account of the small size of the cells lying in a lymphoid reticulum, the myeloid changes in the spleen, the universal distribution of the tumors, and absence of leucocytosis, we regard this case as one of lymphadenia and quite different from the infectious lymphosarcoma. We have encountered one other case of this type in which the liver was chiefly involved. The extensive involvement of the genitals might readily lead to the error of mistaking this disease for the infectious lymphosarcoma.

The above observations, and other peculiarities briefly noted in the literature, emphasize the need of more thorough clinical study of the infectious tumor than it has yet received. Several important problems seem to await solution largely through such clinical study. It is possible that more than one disease has been included in the studies of various observers, and that some conflicting results may therein find an explanation. More minute investigation of the beginnings of the disease may throw important light on the etiological factors. The fully developed spontaneous disease ought to display more clearly the true position of the malady among pathological processes. The relations of the entire group of lymphosarcomata are here brought into question under conditions unusually favorable for their experimental study.

Gross and microscopical appearance of the spontaneous tumors. — The tumors are rather soft, elastic, well-circumscribed masses of grayish white tissue yielding a mucinous fluid on pressure, or they may be firmer and contain more fibrous tissue, especially when regressing. In size they vary from early minute nodules to multiple growths as large as an orange. Caseation is not observed, but large areas in voluminous tumors may necrose. Microscopically, the cells are uniformly large, round or polygonal with pale staining or clear protoplasm and single nuclei with prominent nucleoli and often showing mitotic figures. They much resemble the large proliferating cells of lymph follicles. These cells are arranged not in alveoli, but in cords supported by thin strands of connective tissue, or, in rapidly growing specimens, there are diffuse areas of cells, while in regressing cases the connective tissue may appear in numerous isolated masses of considerable bulk. The blood vessels are moderately developed. Multiplication of cells occurs in all parts of the tumor, producing a growth which displaces the surrounding connective tissue outward as a capsule, which is entirely free from signs of inflammatory reaction.

Our studies have not furnished satisfactory grounds for the exact histological classification of this tumor. While it most resembles a large cell lymphosarcoma, the cells are polygonal more often than round, the fine reticulum of a lymphosarcoma is missing, the growth is much less malignant than large cell lymphosarcoma in man, the arrangement of the cells in alveoli without lumina is sometimes distinct, the protoplasm of the cells is nearly homogeneous, and a mucinous discharge from the cut surface of the tumors is characteristic. The general histological characters suggest to us the diagnosis of alveolar sarcoma or endothelioma. The particular tissue cells giving origin to the tumor have never been determined.

Experimental inoculation. — Nowinsky (1877), who first described the disease in detail, also reported its successful transference by inoculation of the tumors. From an ulcerated

recurrent tumor of one year's growth in the vagina he transplanted pieces to the subcutaneous tissue of three young dogs, and in the course of three or four months saw the development of isolated tumors as large as a walnut or egg. The structure of all the tumors was that of myxosarcoma. No further study of the condition appears to have been made until 1888-9 when Wehr reported the successful transfer of several of the tumors in a series of twenty-four dogs. In one of these, after a year's growth, there were found numerous metastases in the peritoneum and abdominal organs. Since that time the tumor has been studied and numerous successful inoculations practised, by Duplay and Cazin, Geissler, Smith and Washbourn, Powell White, Sanfelice, Sticker, and Bashford, Murray, and Cramer.

The observations of these authors have demonstrated many important facts regarding the inoculability of the process. The animals successfully inoculated have included many breeds, all varieties of dogs appearing to be about equally susceptible. Sticker transferred the tumor from a dog to two foxes, but failed to secure growths in cats, guinea-pigs, rabbits, rats, and mice. Young animals have been chiefly employed, but Smith and Washbourn found that the tumors grew larger in older dogs, and that a moderate proportion of animals possess complete natural immunity.

The course of successful implantations is usually quite uniform. When inserted beneath the skin a period of two or three weeks elapses before any definite enlargement is noted. Thereafter the nodule may steadily increase in size for four or five months, producing a well-circumscribed tumor as large as a hen's egg or small orange. Smaller secondary tumors may develop in the neighborhood, or in the abdomen, spleen, liver, and lung. In progressive cases the tumors reach their largest dimensions in three to six months and the animals die with cachexia and emaciation, often aggravated by sloughing and infection of the larger masses. At any period the tumors may cease to grow and a regressive process begins which ends in a complete atrophy, after which the animals have usually been found

completely immune to reinoculation. Sticker reports spontaneous disappearance of subcutaneous tumors in eighteen out of sixty experimental cases. It has been remarked by Sticker and has been our experience, that while the spontaneous disease often extends through the lymph nodes, the subcutaneous experimental tumors are usually fatal, without affecting the lymph nodes. Geissler, however, saw extensive involvement of peritoneum, abdominal nodes, and spleen, eight months after inoculation of the ventral skin. Sticker observed six cases in which tumors implanted in various localities directly invaded lymph nodes, a course which he referred chiefly to the influence of complicating infections of the tumor. Besides the regional metastases Sticker reports several cases of general sarcomatosis following inoculation, with numerous nodules in the skin, spleen, liver, kidneys, and heart and skeletal muscles, pointing to dissemination through the blood vessels.

Intraperitoneal inoculation was extensively practised by Sticker. Here small multiple tumors of the omentum developed in two months, and in three months diffuse sarcomatosis throughout the peritoneum. Some of his omental tumors reached the size of a cocoanut. Extensive growths followed inoculation of the pleura and tunica vaginalis, while successful implantations were made in the stomach wall, liver, lung, orbit, and bone marrow.

It has been a remarkable feature of the inoculation experiments that by merely rubbing the scarified mucosa with portions of the tumor successful results have been secured. Smith and Washbourn, Duplay and Cazin, and Sticker, all report that after such procedure the inflammatory reaction subsides and in a few weeks single or multiple tumors develop. Complete comminution of the tumor cells destroys the inoculability of the material and filtrates of tumor emulsions passed through paper or earthen filters are innocuous. The tumor cells are still viable after exposure for twenty-four hours at -14° C., but are killed when heated for two hours at 54° C. (Sticker).

The implantation experiments which form the basis for this report were begun in the spring of 1905, and the first plants were made from a tumor of the vagina removed at operation by Dr. Sherwood. A section of the growth used for implantation has in every case been kept for histological examination. These earlier plants were made by two methods, viz.: implantation subcutaneously of a thin section of tumor tissue, and infusion of a suspension of the tumor cells into one of the mesenteric veins.

The tumor pulp was strained through gauze before inoculating. Under these conditions the meshes of the gauze become filled with the gelatinous fragments of tumor tissue, so that only the very finest particles can pass. Such filtrates give much fewer successful inoculations than are obtained when larger particles are included.

The material was brought to the laboratory from the operating room in a special double walled vessel so arranged that a water jacket kept the inner chamber at body temperature. In view of the extraordinary vitality of the tumor cells such a precaution was probably unnecessary. These four earlier experiments made with growths removed from the vagina gave negative results. Previous to the operative removal, one of the tumors from which plants were made had been subjected to treatment by curettage and the application of caustics. It is quite probable that this procedure had interfered with the normal vitality of the cells.

For the later experiments, begun in the fall of 1905, the tumor tissue was obtained from the left inguinal lymph nodes of a five-year-old male Irish terrier. The primary growth was found on the sheath of the penis, but the penis itself was not involved in this case.

A number of dogs were planted with small grafts subcutaneously on the abdomen, others by subcutaneous injection of a suspension of the tumor cells. All of the animals which had been planted by the first method developed tumors in from three to five weeks. The second group were failures. All of the tumors which we have studied experimentally have come from this successful implantation.

The tumor is now in its fifth generation and continues to grow with undiminished vigor. One hundred and twenty-two plants by various methods have been made on twenty-nine dogs of various ages and breeds. From this number thirty-five tumors have developed and there have been twelve spontaneous recoveries.

Certain generalizations may be drawn from the statistics and conditions of these experiments: Old dogs grow the tumor better than young animals. The rate of growth is in a rough way dependent upon the general health of the animal. With poor nutritive conditions the growth is rapid and improvement of health following operative removal of a portion of the tumors is often accompanied by a regression of the remainder. Spontaneous recovery is a common phenomenon, and generally occurs in young, vigorous animals. Four recovered animals we have been unable to infect a second time. In a susceptible animal only a portion of the plants may grow. If one tumor starts to regress, all do. We have never seen some tumors regressing and others growing vigorously in the same animal at the same time.

The crucial point which we have attempted to settle in this study has been the question of the mode of origin of the tumor, *i.e.*, whether the growth comes from the proliferation of the transplanted cells or by stimulation of the surrounding tissue cells, as maintained by Bashford, Murray, and Cramer. The small sections which we have planted for this purpose have been taken from actively-growing tumors and have been planted in other locations on the same animal that furnished the seed. By such procedure we are sure of having a susceptible animal, but in such conditions not every plant will grow.

The small operation wounds healed kindly, and after waiting the desired interval, the plant, together with the surrounding tissue, was removed and preserved for histological study.

It may be a point of considerable importance to note that the operation wounds, from which we afterward removed

tumor grafts, healed in every case by primary union, so that the inflammatory reaction was caused only by the traumatism of the operation. It is readily understood that the particular changes which we wished to observe might easily be obscured by the inflammation following infection.

Histology of the transplanted tumors.— Throughout our entire series of transplantations the structure of the tumor has remained remarkably uniform, agreeing in this respect with the experience of all previous observers. The cells always maintain their large size, round or polygonal form, single nuclei with prominent nucleoli, clear or slightly granular protoplasm with indifferent affinity for eosin (Muller-formol fixation), and arrangement in cords without alveolar lumina, supported by thin strands of connective tissue. Sharp demarcation from the surrounding tissue and absence of round cell infiltration and caseation, are nearly constant features of uncomplicated growths. When the tumors regress there is a focal thickening of the trabecula with simple or degenerative atrophy of the cells, without necrosis. The surrounding tissue is displaced mechanically by the tumor and is usually quite free from signs of inflammation or participation in the growths.

Histological study of the fate of the transplanted cells. — For this purpose pieces of the tumor, implanted in the manner described, were excised with the surrounding tissue at intervals of one to twenty-one days, hardened in Muller-formol, cut in paraffin, and stained by hematoxylin and eosin.

Twenty-four-hour specimens.— Pieces excised after twenty-four hours are found to be lying loosely adherent to the surrounding tissue. On section the nodules are lying in a mass of fibrin, serum, and red blood cells, while polynuclear leucocytes are abundant in the surrounding vessels and blood clot. The compact nodule of tumor tissue is entirely necrotic except for a few islands of ten to fifty intact cells, lying along the periphery of the piece. These islands are clearly shown

to belong to the transplanted piece by their morphology, immediate relations to the necrotic central portions, and separation from the host's tissues by a well-defined layer of fibrin or leucocytes. Not all the cells in these islands are intact. Some are necrosed, others degenerating, most are normal, and a few show mitotic figures (Plate XIX., Figs. 5, 6).

Forty-eight-hour specimens. — After two days the nodules are more firmly adherent, and the only histological change noted is increased edema and diminished leucocytosis in the surrounding tissues of the host. The peripheral islands of tumor cells appear as before and it now becomes evident that these islands are in contact with small blood vessels, apparently those of the original tumor, which contain normal red blood cells as though circulation had become restored in them. The sharp separation of most of the islands from the host's tissues is even more apparent than before (Plate XVIII., Fig. 4).

Three-day specimens. — At this time the implanted piece has become well fused with the subcutaneous tissue of the host, which remains edematous and infiltrated with polynuclear leucocytes. The peripheral islands of intact cells are now usually fused into a definite layer surrounding the necrotic center and are clearly visible to the naked eye in the stained section. In this layer are many blood vessels containing blood which appears to have been in physiological condition. Some of these vessels must have belonged to the original tumor since they are too large to have been developed in three days. The outer edges of the layer of tumor cells are again sharply separated by strands of fibrin from the host's tissues, and we could find no trace of the transformation of fibroblasts or any other cells of the host into tumor cells. At this stage many of the tumor cells show mitosis (Plate XIX., Fig. 7).

Six-day specimens. — The peripheral layer of tumor cells has now increased in depth, while the necrotic central mass has undergone some absorption, and edema and leucocytosis have largely disappeared. The tumor cells have in some segments displaced the host's tissues before them, forming a

thin capsule which separates the tumor from the subcutaneous fat and connective tissue. At other points this separation is less complete and here the tumor cells seem to be mingling with the multiplying fibroblasts of the host. Even in such foci, which are rare, the tumor cells are usually distinguishable from the fibroblasts, and we are unable to see any definite evidence of transformation of fibroblasts into tumor cells. Such areas, however, have doubtless formed the basis of the claim by Bashford, Murray, and Cramer, that the tumor develops exclusively from infected subcutaneous fibroblasts which become transformed into tumor cells. Such areas of mingled fibroblasts and tumor cells are rare in our specimens. All but small portions of the circumference of our implanted nodules are, from the first, sharply separated from the surrounding connective tissues by fibrin, fat, or acellular connective tissue, and in by far the greater part of the growing mass we find it impossible to conceive how the growing cells can be derived from the host's cells (Plate XIX., Fig. 8).

Fifteen days. — At this time the nodule has reached the size of a pea, the necrotic material has been absorbed, the cells are actively multiplying by mitosis. Their arrangement is identical with that of other tumors, and the entire mass is sharply separated from the surrounding tissue by a definite capsule. The surrounding tissue is rather vascular, but exhibits no other signs of inflammation (Plate XX., Fig. 9).

At twenty-one days the appearances have been exactly similar to those found at fifteen days.

Biological studies. — Sanfelice has been the only observer to report positive results from the search for microorganisms in the tumor. He observed in fresh preparations of fragments removed from a vaginal growth refractile double contoured bodies which he regarded as yeasts. These he was able to cultivate on ordinary media, and the culture proved pathogenic for small animals, but caused only inflammatory reactions. Sticker conducted systematic cultures from the growths on various media under aërobic and anaërobic

conditions. The results were negative. Smith and Washbourn, Bashford, Murray, and Cramer, and Sticker, all mention the comparative freedom of the cells from the usual forms of cell inclusions.

On account of the clinical resemblance of the disease to syphilis, we have regularly examined material from the original growths and from the experimental tumors for spirochetæ. Spreads were made from the affected mucous surfaces and from the interior of tumor masses, and stained by Procas or Goldhorn's methods. From some cases the tissue was prepared according to Levaditi's second method for the demonstration of spirochetæ. In seven clinical cases we failed to find any spiral organisms, which seem not to be common saprophytes of the genital organs of dogs. In one large multiple tumor, growing from a transplanted piece, which was sloughing extensively, some large spiral organisms, resembling *Spirocheta refringens*, were found on the necrotic surface and within well-preserved portions of the tumor. In all other cases the results were negative. Spreads from actively-growing tumors are remarkably free from bodies resembling microorganisms, and from the usual cell inclusions of tumors.

NATURE OF THE INFECTIOUS LYMPHOSARCOMA OF DOGS.—The foregoing description of the clinical and pathological characters may form a basis for discussion of the nature of the disease. If the process is an infectious granuloma it is of some practical interest to dog fanciers, and from the standpoint of descriptive comparative pathology, but if it is a true neoplasm, it possesses very great practical and theoretical importance for the general problem of malignant tumors.

From the beginning of the observations on the disease up to the present time opinions regarding the nature of the process have been at variance. Nowinsky and Wehr speak of the growth as a carcinoma, but do not appear to have attempted a careful distinction between carcinoma and sarcoma. Geissler at first regarded his tumor as a carcinoma

on account of the alveolar arrangement (he did not speak of lumina in the alveoli), but the discussion of his communication favored the diagnosis of sarcoma. The description indicates that his specimens were infiltrated with leucocytes. Hansemann, from a cursory examination, pronounced the growths exhibited by Geissler to be granulation tissue or sarcoma. Duplay and Cazin were uncertain as to the nature of their tumor. Smith and Washbourn, Sanfelice, White, and Sticker are certain that their tumors must be regarded histologically as round cell sarcoma. Bashford, Murray, and Cramer believe that they have proven that the process is an infectious granuloma, finding that the transplanted cells necrose while the new tumor grows from the infected cells of the host.

Sticker secured specimens of the tumors studied by Wehr, Geissler, Smith and Washbourn, Sanfelice, and Bashford, and submitted them, with his own, to a large number of German pathologists, all of whom agreed upon the diagnosis of round cell sarcoma.

Supported by this diagnosis of recognized authorities on the structure of tumors, Sticker compares the general features of the disease with malignant tumors in man and shows that on all these grounds the growth must be accepted as a true malignant neoplasm.

(1.) Clinically the other infectious venereal diseases of dogs resembling lymphosarcoma always cause prolonged inflammation and multiple nodules, which are merely enlarged lymph follicles, while inoculation of the vagina by the true tumor produces little or no inflammation, which soon subsides and is followed by solitary, or at best two or three, tumors arising in the subcutaneous tissue.

(2.) Histologically, the tumor is entirely different from any known infectious granuloma, is not accompanied by any inflammatory reaction in the neighboring tissues, and produces metastases which develop from cells carried by the blood stream, and without participation of the tissue cells.

(3.) Experimentally it is necessary to transfer living cells in order to secure a growth, while tumor emulsion

finely comminuted, or strained through paper or porcelain is innocuous.

(4.) The spontaneous transference of the tumor is paralleled by the cases of "cancer á deux," and of epithelioma of the upper transferred from the lower lip in man.

(5.) The spontaneous disappearance of the tumor in dogs is quite comparable to the occasional disappearance of sarcoma and even of carcinoma (Orth) in man.

It must be admitted that Sticker's argument presents a body of evidence which speaks very strongly in favor of the opinion that the infectious lymphosarcoma of dogs is a true tumor. Our experience with the disease fully supports his views, with the possible exception that we are unable as yet to satisfactorily distinguish between his follicular vaginitis and the lymphosarcoma.

Yet, in spite of the opinions of many eminent pathologists based on the histology of the growths, the whole argument appears fatally defective when direct experimental evidence, as furnished by Bashford, Murray, and Cramer, indicates that the transplanted cells die and the tumor grows by infection of other tissue cells. This is the crucial test of an infectious granuloma as against a neoplasm, and if the observations of the English Commission are correct the decision, in our opinion, lies conclusively in their favor. In any event, Bashford, Murray, and Cramer have discerned the true method for determining the nature of the process and the futility of relying upon the histological appearance of the tumors, no matter how experienced the authorities consulted.

Our study of the disease was undertaken with the same object of determining the fate of the transplanted cells and, in spite of preconceived opinions, we had already reached a contrary conclusion when the results of the English Commission came to hand.

As indicated in the description of the changes occurring in the transplanted pieces of tumor, we are unable to confirm the results obtained by Bashford, Murray, and Cramer. In our specimens all the cells of the transplanted pieces do not perish, but a few peripheral islands of cells always remain

alive, showing mitotic division from the first day, and separated from the tissues of the host so clearly as to leave no doubt that the transplanted cells give rise to the tumor. Between the third and fifth days, when, according to the English Commission, all the transplanted cells have perished, our specimens show very active mitosis in a definite layer of tumor cells separated from the surrounding tissue by fibrin. In our experiments the critical time for the transplanted cells comes in the first forty-eight hours when most of the cells necrose, after which mitotic figures in the remaining cells become very numerous.

That some tissue cells may be included in the tumor or be transformed into tumor cells we are not prepared to deny, although there is no definite evidence of such a process in our specimens.

We are unable to explain fully these contrary results of observations on the fate of the cells. It seems possible that Bashford and his associates were dealing with a less active tumor than ours, and with more resistant animals, and that the surviving cells in their experiments were too few to be recognizable as belonging to the transplanted nodule. This suggestion appears the more plausible since these observers state that the transformation of tissue cells into tumor cells is noted chiefly about slowly-growing tumors which are not well encapsulated. Such slowly-growing tumors, however, are less suitable for revealing the true capacities of the cells for independent existence. They admit, also, that in more rapidly-growing tumors the chief part of the growth arises from the tumor cells, while the transformation of fibroblasts is not always in evidence. Yet in progressive infectious granulomata, tissue cells are always being involved in the lesion, and the recorded observations of Bashford, Murray, and Cramer do not seem to fully support their conclusions regarding the nature of the process.

It may be suggested, also, that if an infected tumor were used for transplantation, the inflammation resulting from the bacterial infection might obscure the fate of the transplanted cells.

In view of all the clinical and pathological characters of the disease described by previous observers, many of which we have been able to verify, and of the results of the attempt to trace the fate of the transplanted cells, we are forced to conclude that the infectious lymphosarcoma of dogs is a true malignant neoplasm, of which the cells show, perhaps, a greater capacity for independent existence and infectivity than is seen in any other known tumor process.

The highly infectious nature of the process led Smith and Washbourn to regard the instance of this disease as a powerful argument in favor of the parasitic origin of malignant tumors. If, however, the transplanted cells continue to grow and produce the tumor, then the infectiousness of the process becomes an equally strong argument against a parasitic origin.

Yet the experimental evidence will not permit us to decide positively for or against the parasitic theory. There is the possibility that the tumor cell may contain within itself some form of microorganism which is the source of the peculiar form of stimulation that is the essential factor in the growth activities of the host. We can, however, formulate certain conditions which the hypothetical parasite must follow in this particular instance without committing ourselves to the declaration that such conditions are universal for tumor growth.

We have no knowledge of the parasite outside of the host cell. All those means of comminution which destroy the integrity of the tumor cell stop at the same time its infectiousness. The parasite, if it does exist outside of the host cell, is harmless. Those forms of injury which affect the tumor cell injure at the same time and to the same degree the intracellular parasite. This parallel is particularly interesting with reference to cold. Sticker has found it possible to cool the cells to -14° C. and still have growth. Now -14° C. is that critical temperature which Folin has found for the protoplasm of frog's muscle. If exposed from -15° to -21° C. the muscle goes into rigor on warming, while at -14° C. or above, the tissue is still irritable when thawed.

The critical temperatures of heat and cold of host cell and parasite must be identical. The very careful biological study of the growth indicates that the parasite must be of ultra-microscopic size, or that its protoplasm has such close agreement in staining properties with the tumor cell, and has such an homogeneous arrangement within the latter as to be impossible of discernment by methods now at our disposal. Our only knowledge of the parasite's existence is its supposed responsibility for the growth activities of the tumor. Since the life of the parasite and the manifestation of its activities in producing a malignant growth are absolutely dependent upon the transfer of a living tumor cell from one individual to another, there can be no objection to considering the tumor cell the essential infective agent in the process of transmission. Such conditions imply a symbiotic relation between the two consociating cells that is without analogue in such cases as have already been investigated either in the animal or the vegetable kingdom. The conditions require so intimate and vital a relation between parasite and tumor cell that it is impossible to make any distinction between them.

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DESCRIPTION OF PLATES.

PLATE XVIII. — Fig. 1. Section of original tumor used for transplantation. x 200.

Fig. 2. First day after transplantation. x 320. Island of living cells in edge of transplanted piece. Below is a layer of leucocytes and fibrin.

Fig. 3. First day after transplantation. x 200. Layer of living cells adjoining layer of fibrin and leucocytes, to right, and necrotic tumor, to left.

Fig. 4. Second day after transplantation. x 200. Thin layer of living cells on edge of necrotic tumor, and adjoining fat and fibrous tissue.

PLATE XIX. — Fig. 5. Mitotic nuclei in living cell layer of Fig. 4. x 600.

Fig. 6. Second day. Layer of living cells. x 330.

Fig. 7. Third day. Deeper layer of living cells grading into necrotic tumor, to left, and sharply separated by band of fibrin from host's tissues, to right. x 330.

Fig. 8. Sixth day. Deep layer of growing cells adjoining necrotic tumor above, and edematous tissues of host below. x 200.

PLATE XX. — Fig. 9. Fifteen days. Sharply circumscribed tumor. Compare original piece, Fig. 1. x 330.

Fig. 10. Appearance of whole transplanted piece after three days. On the right the necrotic center is surrounded by a lighter layer of proliferating tumor cells. A dark layer of fibrin surrounds most of the circumference.

Fig. 11. Tumor in situ.

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