BLOOD VESSEL STUDIES ON EXPERIMENTAL FOWL SARCOMA BY INJECTING OPAQUE SUBSTANCE

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I. INTRODUCTION

The study of comparative and experimental tumor pathology is important and of great interest and has already been described by many scholars. I have prepared this paper in order to elucidate further some of the outstanding properties of tumors especially on the blood vessels. By following the usual methods described by my predecessors in the arteriography of malignant tumors, supplementary detail may here be added to the voluminous work on blood vessel studies—this time on fowl sarcoma. There are quite a few reports from SAITO(1–16) and his colleagues, relative to arteriography, which have covered practically the entire field. The last report by SAITO(17) in 1936, at the meeting of the Japan Surgical Association, has given the subject a thorough review in which no stone was left unturned. However, studies on blood vessels of fowl sarcoma are still unheard of, and I am reporting my experimental observations at this time for your consideration. If the findings obtained by my research, were to add to the science of experimental pathology even a ray of light, the time spent in this endeavor is not in vain.

Before proceeding it may not be amiss to tell briefly the literature on transplantable malignant tumors and arteriography.

II. LITERATURE ON TANSPLANTABLE MALIGNANT TUMORS

There are quite a few transplantable malignant tumors of animals on record. HANAU(18) [1889], was the first man to succeed in the

transplanting of mouse cancer to the same species. Forty-nine years have elapsed since this discovery, and in the mean time numerous malignant tumors have been discovered among animals. On mice, we have such men as MORAN(19), JENSEN(20), EHRLICH(21), TSUTOI(22), YAMAMOTO(23), HIGUCHI(24); on rats, we have names like JENSEN(20), MICHAELIS(25), LEWIN(26), FLEXNER(27), JOBLING(27), LOEB(28), HAYASHI(29), FUJINAWA(30), UMEHARA(31), KOKITA(32); on rabbits, SCHULZE(33) and KATO(34). On guinea pigs, LUBARSCH(35) and SUEYASU(36) may be mentioned. On dogs, STICKER(37), NOVINSKY(38), BASHFORD(39), MURRAY(40), MATSUI(41), YAMAGIWA(42) and YAMAMOTO(44) may be mentioned. On fowls, the names of ROUS(45), TYTLER(46),(47), FUJINAMI(48),(49), INAMOTO(50), HAYASHI(51), BUTO (52), KATO(53) and OSHIMA(54) are outstanding. On pigeons, UMEHARA(31) may be mentioned. In regard to artificial production of tumors, we may mention FIBIEGER(60),(61), as the first man to produce cancer in the mouse. He was followed by such men as YAMAGIWA(55), (56),(57), ICHIKAWA(58) and TSUTOI(59), who have produced cancer by painting tar on the mouse.

We are concerned here more or less with the subject of malignant tumors of the fowl. The fowl sarcoma was discovered by ROUS(45) in America, and FUJIINAMI(48) in Japan. This type of sarcoma since its discovery has been used in Japan for various studies by many scholars; more so in this country than in other countries. There are about 14 or more similar stocks of fowl sarcoma propagated by various men at the present time. FUJINAMI(48) and INAMOTO(50) first described in 1910 a spontaneous connective tissue neoplasm of the hen, probably a myxoma or a myxo-sarcoma, which was so readily transplantable that inoculation had been almost invariably successful.

In performing my experiment, I have through the courtesy of the pathological department of this University, used the sarcoma generally known as "OSHIMA fowl sarcoma" in Japan. This type is characterized by being very mild in malignancy and has been used quite often for experimental purposes. By a small fragment transplantation, the graft becomes united after two weeks, and within one to two months the host dies due to the enlargement of the neoplasm. The development of the tumor is either characterized by expansive proliferation or by infiltration. However, it is subject to fluctuations in growth energy and may grow progressively, or disappear spontaneously by absorption. Accord-

ing to OSHIMA(112), histologically it is typical spindle-celled saroma, and tumors resulting from grafts always showed essentially the same sarcoma structure varied between that of a pure sarcoma and that of a myxosarcoma.

III. LITERATURE ON ARTERIOGRAPHY

One year after the Roentgen ray was discovered in 1895, GRUNMACH(62), on a fifty-six year old arteriosclerotic patient, noticed a faint shadow of the radial artery in an X-ray picture, and ascribed it to deposits of carbonates. This probably was the first study on blood vessels with X-ray. Arteriosclerotic vessels by X-ray were studied since then by many others. SCHEPELMANN(63) [1910–1925] injected into the blood vessels several opaque substances and took X-ray pictures of them but failed to obtain satisfactory arteriograms. He used mainly heavy metal alloids and oil. He was followed by others who injected opaque substances for arteriographic purposes. I shall mention only the names of these research workers in the chronological order.

Name and year.	Material used.
SCHEPELMANN [1910](63)	Mercury
FRANK und ALWENS [1910](64)	„ and bismuth
CAMEERON [1918](65)	Iodide and bromide
RYOZO ETO [1919](66)	„ „
CARZIER [1920](67)	Iodized oil
BERIBERICH und HIRSCH [1923](68)	Iodide and bromide
DUNNER und CALM [1923](69)	„ „
BROOKS [1924](70)	„ „
MONIZ [1927](71)	„ „
SINGLETON [1928](72)	„ „
CARBONNEL et MASSE [1924](73)	„ „
REYNALD des SANTOS [1929](74), (75)	„ „
LEIBOVICI [1927](76)	„ „
MAUCLAIRE [1927](77)	„ „
METIVET [1929](78)	„ „
SAITO, KAMIKAWA, YANAGIZAWA [1929] (1), (2), (3), (80), (79)	„ „
AMANDIO PINTO [1930](80)	„ „
TANABE and KAMITATE [1930](82)	„ „
E. MONIZ [1927](71)	Tetraiodophenolphthalein and tetrachlophthalein

Name and year.	Material used.
BERIBERICH und HIRSCH [1923](68)	Calcium lactate and chloride
„ „ „ (68)	Calcium iodide
„ „ „ (68)	"Dominal X"
SICARD [1923], SICARD et FORESTIER [1923](83)	Iodized oil
SICARD, de GENNES et COSTE [1924](84)	„ „
BAILLAT et LAPASSET [1926](85)	„ „
DESPLAT, DESBONNETS, BUISINE et DELCOUR [1926](86)	„ „
BAILLAT et MERIEL [1926](87)	„ „
HARVIER et LEMAIRE [1927](88)	„ „
CARNETT et GREENBAUM [1927](89)	„ „
SICARD et HAGUENAU [1928](90)	„ „
LEIBOVICI [1929](76)	„ „
FRAZIER [1929](91)	„ „
SAITO, KAMIKAWA, YANAGIZAWA [1929] (124), (125)	Iodized oil emulsion (L'Ombre)
MARTIN [1929]	Iron chloride
DEMEL, KOLLERT und SGALITZER [1930] (92)	"Uroselectan", "Abrodil"
SAITO and KAMIKAWA [1930](6)	„
„ „ [1932](11)	Thorium sol
CHARDONNEL [1931](93)	„
SCHULLER [1931](94)	Abrodil
SUGII [1930](95)	"Sugiuron"

In going over the literature on the studies relating to the nutritive vessels of neoplasm, I find quite a few have already reported in detail. DANIELS(96)[1991], BORST(97)[1902], RIBBERT(98),(99)[1904], and TANAKA(100)[1908], have studied the microscopic sections. FREUND (101)[1904] was the first one to observe the structural changes of the nutritive vessels of neoplasm. By blood vessel injections, he studied on normal and abnormal uterus, whereby he was able to confirm five fibroids in a diseased uterus. SAMPSON(102) in 1912 injected into the blood vessels of 100 cases of fibroids of uterus, dyes as well as lead perparations and took X-ray pictures. He reported his findings on the nutritive blood vessels in comparison with that of the normal uterine vessels. MONIZ(71), in 1927 injected sodium iodide into the vessels of the brain and obtained X-ray pictures of the cerebral tumors. SAITO (103),(124), in 1927, likewise took X-ray pictures of brain vessels in cases of brain tumors. In 1930, after improving his technic, he used

oil emulsion of iodide, and took satisfactory brain arteriograms in which he noted the multiplication and enlargement of the vessels in case of brain neoplasm. Furthermore, he reported on the blood vessel findings in cases of cancer and non-malignant tumors elsewhere than that of the brain. Later, REYNALD dos SANTOS, A. C. LAMAS et J. P. CALDAS(104) [1931], reported on blood vessel findings on sarcoma of the extremities. Again SAITO(105)[1933] reported on 20 cases of sarcoma of the extremities by taking blood vessel X-rays, and discussed in detail its findings and value from the clinical standpoints, particularly in the diagnosis of such tumors.

Blood vessel studies on transplantable tumors on animals were done by SAITO and his colleagues (HASEGAWA, KANNO ,ASAI, CHIN). HASEGAWA(106)[1933], studied rabbit's sarcoma (Subcutaneous growth); KANNO(107)[1934], studied likewise rabbit's sarcoma, and the blood vessels of the internal organs; ASAI (108)[1936], likewise studied rabbit's sarcoma transplanted in the lungs; CHIN(109), (110)[1937], studied experimentally, sarcoma of the blood vessels of rabbits brains. Finally SAITO(16), in 1934, clinically studied malignant tumors before and after Roentgenological treatments, and noted the changes in the blood vessel picture in respect to the nutritive blood vessels as well as the neighboring or the surrounding blood vessels. K. FUTAGAMI(126) [1936] studied blood vessels of rats' sarcoma and cancer.

IV. METHOD OF EXPERIMENT AND MATERIAL USED

I have used for this experiment white leghorn hens, ranging from 1 500 to 2 000 grams in weight. Before experimentation, they were placed in the chicken coop and fed for one week with mixed grains, vegetables, etc. in order to accustom them to the new environment.

In order to accomplish grafting, the fragment of tissue about the size of millet seed consisting of living tumor cells along with the framework on connective tissue and blood vessels was usually placed under the skin and closed by suture. It usually manifested itself into a very well defined tumor when it established a foothold on the host. In addition to the above method, it was transplanted by injecting the tumor emulsion. In accomplishing this, the tumor tissue was minced finely with scissors, and then the resultant emulsion was taken up by a syringe and needle with a little amount of saline and injected into the desired tissue or vein.

After a week or so from the date of the grafting, the animal was bled to death by severing the carotid artery in the neck. Then all the feathers were removed without injuring the skin which is apt to be torn. After a careful scrutiny of the different parts of the organs in which the tumor had taken its foothold, the opaque substance was injected in sufficient amount into the proper arteries. The X-rays were taken after isolating the desired organs.

Injection materials. There are quite a few injection materials which may be used in arteriography. However, a careful selection of the material is of importance if any success is to be obtained. Thorotrast, a preparation of thoriumdioxydsol with 25% ThO_2, is univers ally used in the arteriography of the extremities and brain but is very expensive. It is used especially in taking arteriograms of living humans as well as in living animals. According to ASAI(108), he has failed in taking good pictures of the lung in rabbits. TAMANA(111) also failed in taking vessels of the lungs in animals.

Those injected after the death of the animal for blood vessel visualization are the cinnabar, a red mercuric sulphide, and umdrathor. I have used in my studies for arteriographic purposes in the fowl sarcoma, either umbrathor or cinnabar. HASEGAWA (106), KANNO (107), ASAI(108) and CHIN (110) have used umbrathor and reported great successes in their researches. This so called umbrathor is a light milky emulsion of a 25% solution of the thoriumdioxydsol(mit Schleimhauten gelatinierendes Thoriumdioxydsol mit 25% ThO_2), produced by Chemische Fabrik von Heyden Aktien gesellschaft, Radebeul, Dresden, Germany. Originally this preparation was used for X-ray pictures taking of the mucous membrane of the stomach and bladder, but was very useful in taking pictures of small vessels. However by injecting into the living animals, it causes convulsions and death. If used after death of the animal, it gives excellent pictures. Even if by chance the injected solutions escape, they fix themself on the endothelium of the blood vessels in sufficient quantities to serve the purpose of arteriography. Therefore, by removing the unnecessary organs that will confuse the interpretation of the shadow, excellent pictures of the desired vessels are possible for X-ray purposes.

The cinnabar has been used widely in the past in dead animals for X-ray purposes. For blood vessel visualization, it is an excellent medium if properly prepared and handled. CHIN (110) in 1938,

reported excellent results for taking blood vessels of the brain tumors (experimental) of the rabbits with Chinese cinnabar. He prepared the cinnabar by mixing with gelatin and Leim Injektion Masse, a preparation, sold by Maruzen Drug Co. I have prepared my cinnabar without the said lime. After finely pulverizing the cinnabar in a mortar with a pestle, I have added gelatin (8 g. which was dissolved in 100 cc of hot water) to make an emulsion of about 30% (cinnabar). To accomplish this, it was boiled, and the constituent particles were thoroughly stirred, after which it was filtered with a gauze. The filtrate was then preserved in the refrigerator. When in need it was boiled to about 40 degrees centigrade, and a sufficient amount was injected into the blood vessels. X-rays were taken after solidification in the vessels.

V. PATHOLOGY OF FOWL SARCOMA

Only a brief account of the tumor will be recorded here. A detail account of this tumor was described by OSHIMA (112), (113) in the Aichi Medical Journal in 1924. If the tumor is grafted into the breast of the fowl, there is a slight difference in the growth depending on the type and condition of the host but more or less similarity is encountered. It takes about ten days to two weeks before it is recognizable. When the tumor is well established and grown for 14 days, it is about the size of a finger tip. In general the neoplastic tissue is greyish white with fine striation on the cut surface. More often it is soft, greyish pink, translucent and gelatinous. In the last instance, it contains much mucoid material. The surrounding tissue is congested and shows signs of marked vascularization.

As the tumor grows, there may appear central coagulative necrosis, or a cystic change takes place frequently, caused as the result of hemorrhage. The cysts are often light chocolate color and contain ropy fluid. At about 3 weeks, the mass is about the size of the tip of a thumb. The skin will be distended, and on the surface there will be seen dilated blood vessels. A tumor of such character grows very fast, and in about 4 weeks it enlarges to the size of a hen's egg. After one month and a half, the fowl shows signs of weakness. At this stage the tissue of the host is markedly infiltrated.

On section, it is seen that the mass extends to the muscle sheath by expansive invasion; proliferating strands of tumor tissue extend into

the normal structure utilizing for their support the blood vessels and connective tissue already present. Those tumors which have grown to a large mass show a flat surface and a peripheral congestion. Usually the central portion of the tumor shows necrosis of cystic degeneration. Considerable amount of mucin may be present in the central portion and may be circumscribed by nodular masses of tumor. According to OSHIMA(112), at times there may be a scanty amount of mucoid material.

The histological study of the tumor in the early stages is best made by fragment grafting. During the first forty-eight hours, the tumor fragment is unchanged but surrounded by polymorphonuclear leukocytes. Later fibroblasts and macrophages make their appearance.

WOGLORN(114), describes the histological picture of the tumor on the third day as follows: "During the 3rd day the graft often united at one or more points with the host's tissue, and although it still remained unvascularized, proliferation was in active progress, cell division taking place chiefly by amitosis, and the fragment was not infrequently much increased in size. It's loose structure facilitated nourishment by a direct interchange of fluids but unless the graft were broken or of very small size necrosis took place at its center. The polys commenced to disappear at this time and fibroblasts, macrophages and an abundant supply of newly formed capillaries took their place."

As the tumor enlarges the peripheral portion is noticed to accomplish vascularization and multiplication of cells takes place. The activity of the segmentation of the neoplastic cells is startling. For a time there may be no formation of blood vessels, and in such cases the growth of the tumor is very slow. There are proliferating strands of spindle cells invading the normal tissue with round cells and leukocytes mingled among them. In general the peripheral portions are abundant with cells and comparatively less mucoid substance. Occasionally, one may find an increase in the fibroblasts and young connective tissue, depicting a histological picture of a granulation tissue. Such a condition is more or less found in young or weak fowl.

At first the tumor is encapsulated with a thin membrane, but as it rapidly proliferates in strands, the adjacent tissues are seen to disappear. This tumor, according to OSHIMA(112) and his colleagues, has the characteristic of being absorbed to the extent of 18%.

Metastases which occur by way of the blood stream, and but rarely through the lymphatics, are rarely seen. I have encountered only a few out of 60 transplanted cases. OSHIMA(112) found only 9 in 168 cases. He reported that when there are metastases, they are usually encountered with a bleeding type of tumor, which may mean that there is relationship of the bleeding tumor to that of metastases. The histological picture of metastases depends a great deal on the organ. However, there is more or less tendency to form in a nodular mass, and a definite demarcation with the surrounding tissue. The color is greyish white and resembles the original tumor. The microscopic picture is the same as that of the original tumor. Occasionally the blood vessels are plugged with tumor cells and continuous strands of tumor tissue appear in the normal tissue. To a considerable extent in cases of metastases, we find tumor cells invading the blood vessels. The secondary growths in the viscera are first evident as small globular masses of cells with a blood vessel in the midst, occluded by tumor tissue.

VI. THE BLOOD VESSELS OF THE FOWL

Before proceeding it is necessary to familiarize ourselves briefly with the normal blood vessels of the fowl. The fowl blood vascular system consists of the heart, the arteries, the veins, and the capillaries. I shall review only the arteries which had to do with my experiments. The common aorta in the fowl is short, and it originates to the right of the inferior bodies of the vertebrate, between the right bronchus and the right lung. It gives off the right and left brachiocephalic arteries which subdivide into truncus caroticus and subclavia. We are concerned here with the subclavia which is the trunk for the sterno-clavicularis, axillaris and thoracalis.

The axillary artery extends out of the thoracic cavity along with the brachial nerve plexus. It gives off the subscapularis which supplies the muscles of the scapular region. It continues as the brachialis. The brachialis gives off the anterior humeral circumflex artery and the brachialis profunda artery. Near the elbow joint, it gives off the ulnar and radial arteries (Fig. 1 & 2).

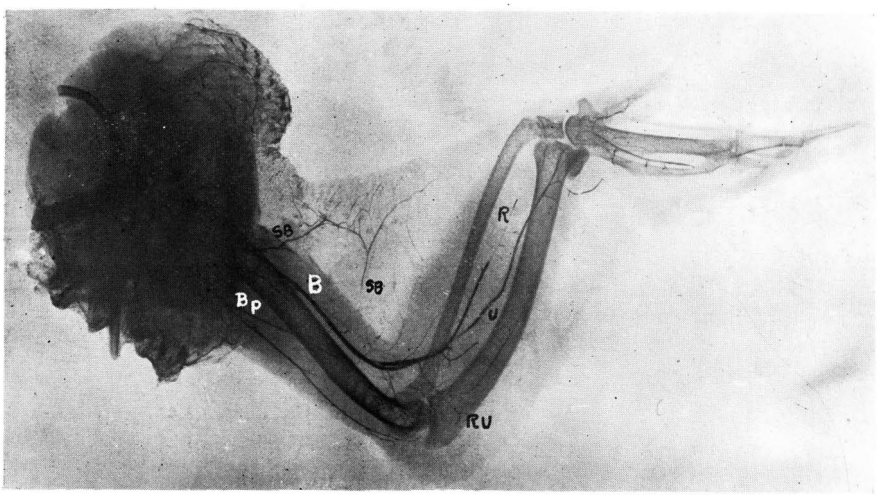

Fig. 1. Arteriogram of the forelimb of the fowl after injection of cinnabar into the brachiocephalic artery. The limb was severed after the injection.

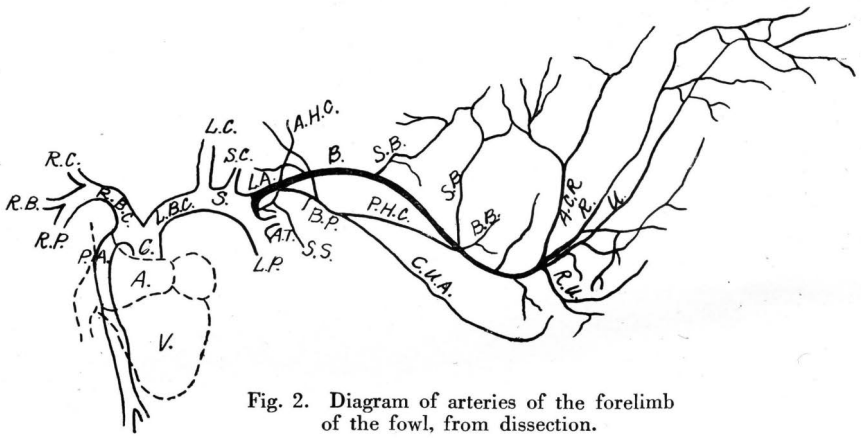

Fig. 2. Diagram of arteries of the forelimb of the fowl, from dissection.

V. Ventricle of heart
A. Auricle
C. Common carotid artery
R. B. C. Right brachio-cephalic artery
R. C. Right carotid artery
R. B. Right brachial „
R. P. Right pectoral „
P. A. Posterior aorta
L. B. C. Left brachio-cephalic artery
I. C. Left carotid
S. Subclavia
S. C. Sterno-clavicular
L. A. Left axillary artery
A. T. Anterior thoracic artery
L. P. Left pectoral artery
S. S. Sub-scapular artery
B. Brachial artery
A. H. C. Anterior humeral circumflex artery
B. P. Brachial profunda artery
P. H. C. Posterior humeral circumflex artery
C. U. A. Collateral ulnar artery
S. B. Skin branches
B. B. Biceps branch
A. C. R. Anterior collateral radial artery
R. Radial artery
U. Ulna artery
R. U. Recurrent ulna artery

The brachialis profunda artery supplies the muscles of the posterior part of the humerus, the skin of the wing, and the muscles of the brachial region. It subdivides into the circumflex humeralis posterior, the collateral ulnar artery and the collateral radialis.

The radial artery passes downward on the outer rim of the forearm. It continues down to the carpal region and gives off the recurrent radial, and passes on to the middle finger, terminating by anastomosing with the collateral radialis artery.

The ulnar artery passes downward to the carpal region along the inner surface of the ulnar bone. It divides into smaller and larger branches. The smaller supplies blood to the thumb. The larger lies between the second and the third finger bones, and passes to the flexor side of the hand and extends to the last finger-joint. The recurrent ulnar passes backward to the olecranon of the ulna, and supplies the muscles, the skin, the feathers and other structures of the region.

Visceral blood vessels. In order to familiarize ourselves with the blood-vessels of the visceral organs, I shall review here briefly the essential vessels. The celiac axis, which is the main trunk for the abdominal organs, is given off from the descending aorta originating near the seventh sternal vertebra. It gives off three main branches, anterior, posterior, and the recurrent intestinalis under the left lobe of the liver, and along the left side of the stomach. The posterior or recurrent sinister gives arterial branches to the proventriculus, the gizzard, the pyloris, and the left lobe of the liver. The anterior or recurrent dexter gives off a renal artery and from four to six splenic arteries.

The recurrent intestinalis artery is located within the duodenal loop, and supplies the duodenum and the pancreas. It subsides to form the recurrent ilio-colicus which supplies the large intestines including the caeca.

The celiac axis also gives a branch called arteria hepatica dextra and supplies several branches to the right lobes of the liver and to the gall-bladder. The muscle of the stomach is also supplied by the gastric branch. The anterior and posterior recurrent branches often anastomose.

The anterior mesenteric artery originates from the posterior aorta. It divides into many branches, which form the recurrent branches, and in turn form the mesenteric arches. These arches give off branches

to supply the intestinal walls. The anterior mesenteric artery also gives off recurrent ilio-celiacus which supplies the caeca. The other branches are distributed to the small intestine and end in the recurrent superior hemorrhoidal arteries.

The posterior mesenteric artery originates from the posterior aorta near the arteries of the thigh and is distributed to the large intestine. It anastomoses with the superior hemorrhoidal arteries. It also gives off a branch to the caeca.

Arteries of the lower extremities. The posterior aorta gives off in pairs the crural arteries (external iliac). They pass through the lobes of the kidneys and extends out of the pelvic cavity in front of the spine. The three branches, namely, the internal pelvic (umbilical), arterior circumflex femoris, and the femoral artery are given off. The internal pelvic is given off just as the cruralis leaves the pelvis. It extends into the umbilical region, by passing on the inner surface of the lumbosacral bones, by supplying blood to the obturator internus muscle. The anterior circumflex femoris extends dorsalward, after passing between

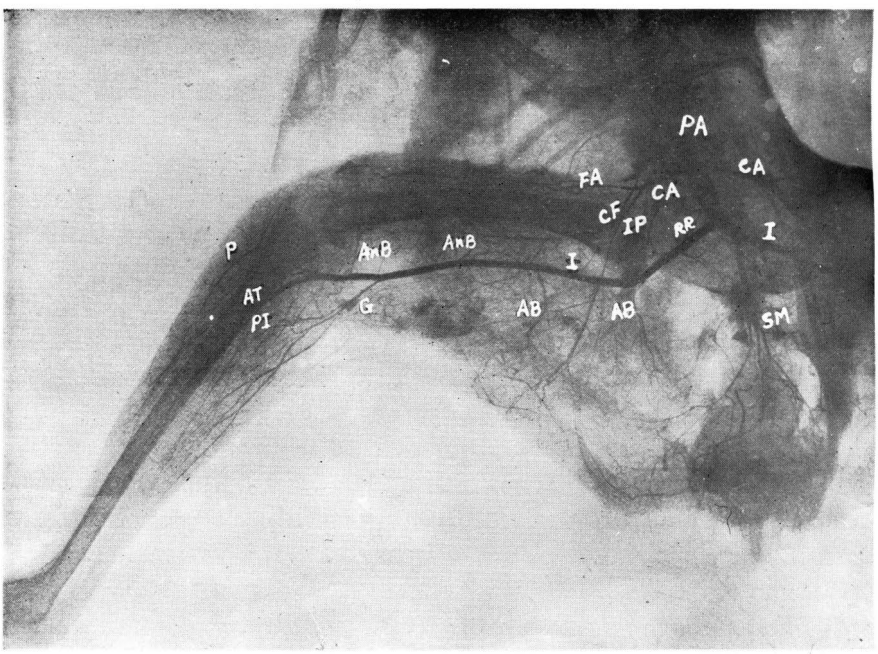

Fig. 3. Arteriogram of the lower extremity of the fowl after injection of cinnabar into the abdominal aorta. The names of the arteries are given in Fig. 4.

Fig. 4. Diagram of arteries of the lower extremity of the fowl after injection of cinnabar into the abdominal aorta.

P. A.	Posterior aorta	*A. B.*	Adductor branch artery
C. A.	Crural artery	*An. B.*	Anastamotic branch artery
I. P.	Internal pelvic artery (umbilical)	*G.*	Gastrocnemius artery
C. F.	Circumflex femoral artery	*P. T.*	Posterior tibial artery
F. A.	Femoral artery	*A. T.*	Anterior tibial artery
I.	Ischiaidc artery	*P.*	Peroneal artery
R. R.	Recurrent renalis		

the sartorius and vastus internus, by supplying blood to them, the vasti muscles, and the ilio-trochanteric region. The femoral artery passes beside the vena cruralis and extends down the posterior surface of the thigh to the knee joint, supplying the upper thigh muscles, except the adductor muscles (Fig. 3 & 4).

The ischiadic artery is the main artery of the posterior extremity. It is given off from the posterior aorta and passes between the main lobes of the kidney. On its way outward from the pelvis, it gives off a recurrent renalis which supplies the kidney, and on the left side, a branch to the oviduct and to the ligament of the oviduct. After passing through the oviduct, branches to the adductor muscles of the upper and lower thigh are given off. The anastamotic branches are also given off to unite with the femoral artery. Before terminating in the anterior and posterior tibial arteries, it gives off two branches at the knee joint and also to the gastrocnemius, and extends below the intertarsal joint.

The tibialis antica (anterior tibial artery), as it runs downward, gives off branches to the knee joint, and also the peroneal artery, which passes through the membrana interossea. It gives branches to the anterior side of the lower thigh and terminates in the anterior tibial plexus.

The tibialis posticus, which is often smaller than that of tibialis anticus, passes between the gastrocnemius and the deep flexor muscles. It gives off branches in its course to the skin and to other parts, and disappears below the intertarsal joint.

The above mentioned blood-vessels of the fowl have been studied by injecting cinnabar, and carefully dissected out and identified. For detailed studies of the circulatory system of the fowl, the author wishes to refer the excellent text of KAUPP(115)—"The Anatomy of the Domestic Fowl".

VII. ARTERIOGRAPHY ON SARCOMA TRANSPLANTED INTO THE SUBCUTANEOUS TISSUE OF THE FORELIMB

Case I. No. 38, white leghorn hen. A fragment of sarcoma was transplanted into the subcutaneous tissue of the left wing, after making a rent in the skin without injuring the visible vessels. On the 12th day the fowl was killed. The grafted sarcoma was about 1 cm in diameter, nodular, and slightly elevated above the skin; the central portion was bluish black and necrotic. After the feathers were entirely removed, the chest was opened, and from the left brachio-ceptalic artery, 3 cc of umbrathor was injected. After severing the left forelimb from the trunk, an X-ray was taken.

The arteriogram (Fig. 5) reveals that the central portion is devoid of malignant tissue, showing a small white spot (necrotic). This central area, about $\frac{1}{2}$ cm in diameter, is also devoid of blood vessels. Circumscribing the tumor, there are markedly irregular blood vessels as if enfolding the tumor mass. These vessels are slightly larger, as shown by the heavy shadow cast, than that of the neighboring vessels. They are formed by the branches of the skin vessels which are given off from the brachial and the radial arteries. These skin branches (Fig. 5 *A*, *B*, *C*, *D*) may be called the nutrient vessels of the sarcoma in this case. It is not possible to classify the nutrient vessels as to primary or secondary in this case, as SAITO(17) reports in 1936. The arterial trunks that give off the cutaneous branches do not show any changes in the size, course, ind location. The circumscribed blood vessels in this case are

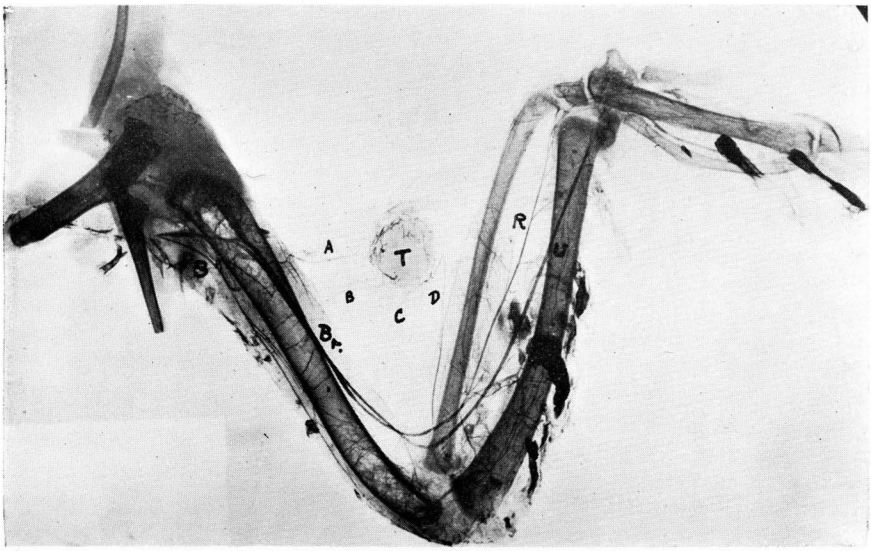

Fig. 5. Arteriogram of sarcoma transplanted into the subcutaneous tissue of the wing. *T*, tumor; *P.B.*, Profunda brachialis; *R*, radial artery; *U*, ulnar artery; *A, B, C, D*, cutaneous branch arteries feeding the tumor; *Br*, brachialis.

seen to obtain their blood supply from four sources wihch may be designated as *A, B, C, D*, cutaneous branches. The vessels from these four sources seem to anastomose as they enclose the tumor. It is evident by the absence of the blood vessels in the central portion that the tumor is nourished from the peripheral area.

The enlarged figure of the tumor area (Fig. 6) reveals a slight amount of small blood vessels circumscribing the neoplasm. At this stage of the tumor growth, the peripheral blood vessels are comparatively less, when compared to that of a long standing case. However, it is certain that the encroaching blood-vessels are slightly larger and more tortuous than those of the adjacent vessels.

Upon section, the tumor is necrosed in the central portion, showing old blood accumulation, and only a slight amount of chocolate colored mucoid substance. The peripheral portion of the tumor is vascular, and fresh tumor tissue is present. The section, microscopically is represented by a massive accumulation of spindle cells, arranged in bundles with a slight vascularizing frameworks (Fig. 7 & 8). There is considerable variation in the size of the cells and in the staining quali-

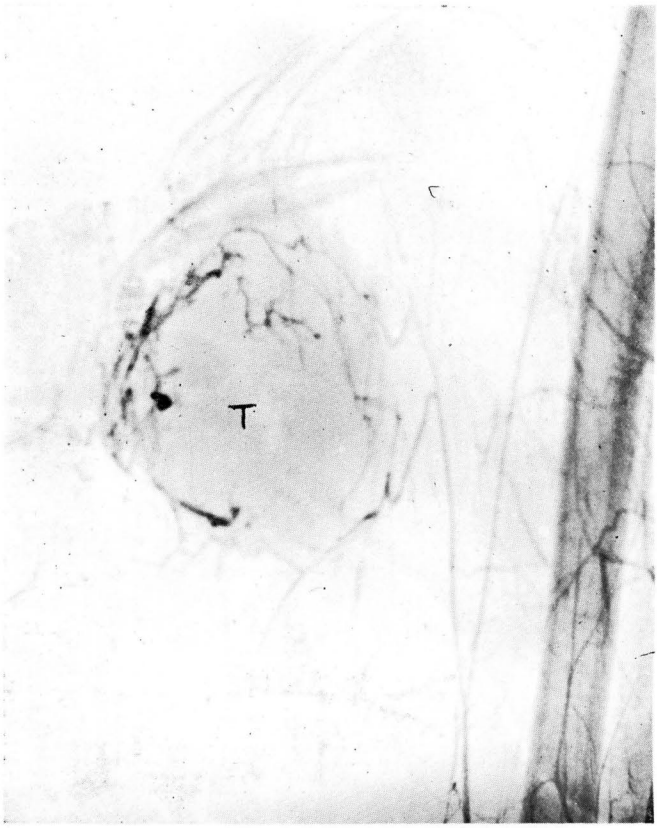

Fig. 6. Enlarged photo of Figure 5. *T*, tumor circumscribed by net work of blood vessels.

ties of the nuclei. The proliferating strands of spindle cells are seen extending into the normal structures.

Case II. No. 21, white leghorn hen. A fragment of sarcoma tissue was transplanted in the subcutaneous tissue of the under surface of left wing. The fowl was killed 22 days later as usual by bleeding, and $1\frac{1}{2}$ cc of umbrathor was injected into the left brachio-cephalic artery and an X-ray taken (Fig. 9).

The arteriogram shows the blood vessels are encroaching on the tumor and enfolding it very plainly. As usual the central portion is devoid of blood vessels. The nutrient vessels are formed by three main sources which are apparently the muscular branches of the brachialis. These three branches are very markedly enlarged, taking a

Fig. 7. Low power photomicrograph showing bundles of tumor tissue with scanty blood vessels.

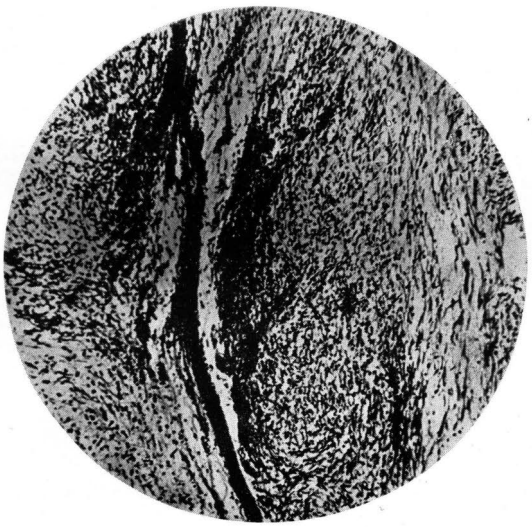

Fig. 8. High power photomicrograph of Fig. 7. Note the spindle cells.

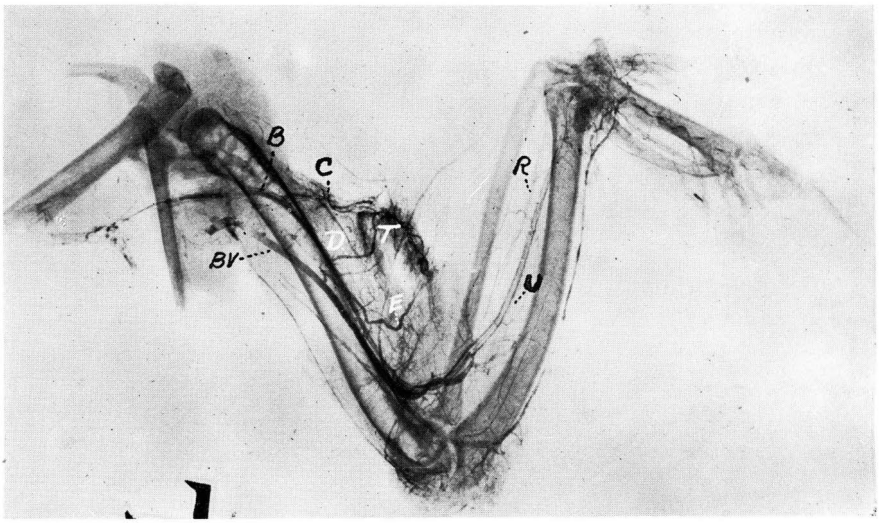

Fig. 9. Arteriogram of sacoma transplanted into the subcutaneous tissue of the wing (Case II). *T*, tumor with central necrosis; *B*, brachial artery; *B. V.*, brachial vein; *C, D, E*, muscle branches of brachial; *R*, radial artery; *U*, ulnar artery.

tortuous course toward the tumor. Upon circumscribing the tumor, an irregular net work is produced. Aside from these main sources of blood supply, the cutaneous branches from the brachialis take part in supplying the blood to the tumor. They are also enlarged and somewhat displaced. Since the tumor is larger than that of Case I (Fig. 5), apparently due to pressure, the umbrathor does not show well in the radial and ulnar arteries. In addition, the umbrathor has entered the brachial vein (Fig. 9 *B. V.*), as well as the minute veins surrounding the tumor. The entrance of the opaque media into the brachial vein is no doubt due to the pressure of the tumor over the distal portion of the brachial artery.

The tumor was the size of a tip of the thumb and more or less forming a globular elevation under the skin (Fig. 10). The central portion was necrotic and soft. The cut section showed a superficial tumor with central necrosis. There was no involvement of the muscle. Microscopically there was a vascular congestion in the periphery of the tumor.

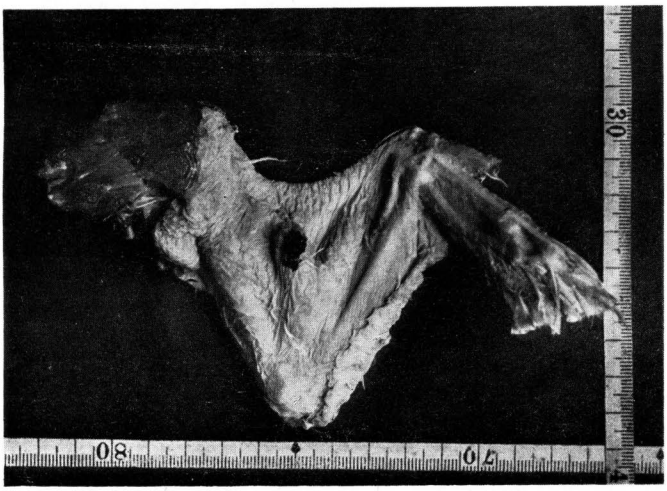

Fig. 10. Sarcoma grafted under the skin of the under surface of the left wing (Case II). Note the central necrosis.

Case III. No. 20, white leghorn hen. This was a two-week old transplanted sarcoma of the right wing. It showed an oval growth, a little smaller than a pigeon's egg, elevated above the skin. The tumor

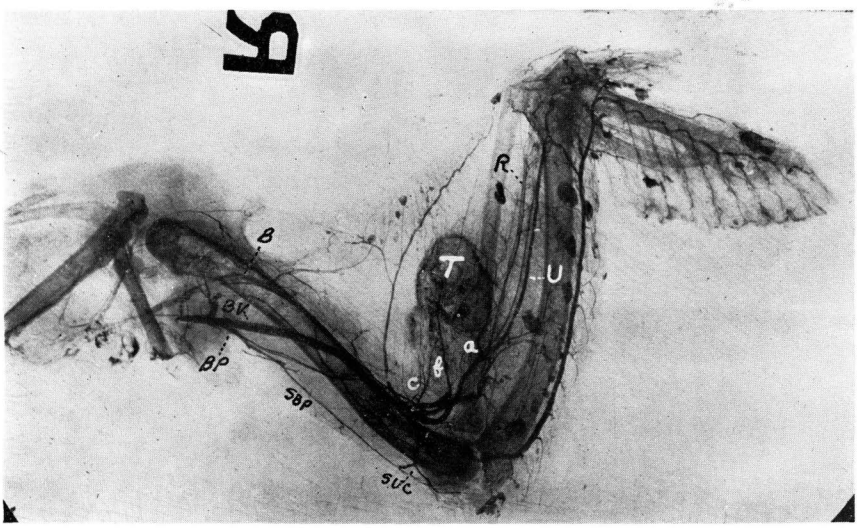

Fig. 11. Arteriogram of sarcoma transplanted into the subcutaneous tissue of the right wing (Case III). Note the vessels surrounding the tumor. *B*, brachial artery; *B. V*, brachial vein; *B. P.* brachial profunda; *S. B. P*, superficial branch of *BP*; *S. U. C*, superior ulnar collateral; T, tumor; *R*, radial; *U*, ulnar; *S. B*, skin branch; *a*, *b*, *c*, nutrient vessels to tumor;

was soft in consistency. Later upon section it was found to be gelatinous in the central portion with mucoid substance. There was no infiltration into the muscles. As usual 2 cc of the umbrathor was injected into the right brachio-cephalic artery and an X-ray taken.

The arteriogram (Fig. 11) shows an oval growth, $1\frac{1}{2}$ cm $\times$ 2 cm in diameter. The tumor is encircled by thin blood vessels which are not very plain in view. The central portion of the tumor shows three or four spots casting heavy shadows. These are accounted for as hemorrhagic areas in the tumor. The nutritional sources of the tumor are mainly from three cutaneous branches of the radial artery. These branches are as usual enlarged. The nutritional branch which is nearest to the tumor is the largest of them all. Due to the pressure caused by the tumor, the ulnar and radial arteries take a curved course for a short distance from the brachial bifurcation.

Case IV. No. 16, white leghorn hen. Tumor fragment was transplanted 22 days before and showed a round growth about the size of a pigeon's egg on the under surface of the right wing. The elevated surface measured $2\frac{1}{2}$ cm $\times$ 2 cm in diameter. In the upper central portion

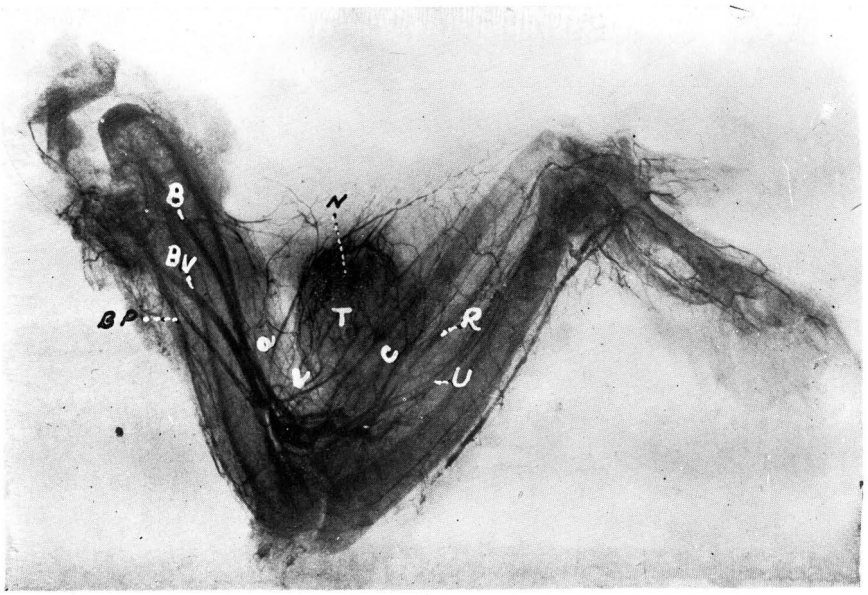

Fig. 12. Arteriogram of sarcoma transplanted into the subcutaneous tissue of the right wing (Case IV). *T*, tumor with central necrosis *N*; *a*, *b*, *c*, blood vessel branches feeding the tumor: *B*, brachial artery; *B. V*, brachial vein; *B. P*, brachial profunda artery; *R*, radial artery; *U*, ulnar artery.

there was a small area of necrosis surrounded with chocolate colored mucoid substance. The muscle tissue was not involved. Upon killing the fowl, $2\frac{1}{2}$ cc of umbrathor was injected into the right brachio-cephalic artery and an X-ray taken (Fig. 12).

The arteriogram (Fig. 12) presents a round growth with a mass of blood-vessel net-work covering about $\frac{3}{4}$ of the surface; only a portion of the upper area is devoid of the net-work. Again the tumor, as in the former cases, derived its blood supply from three main sources; i. e., two branches from the brachial and one from the radial. However, there are many small arteries which join with these main sources and act as nutritional vessels. These vessels encroach the tumor, and a massive net-work is in evidence. This is especially true when the

Fig. 13. Enlarged view of Fig. 12. Note the ramifying blood vessels encircling the tumor.

arteriogram is enlarged (Fig. 13). The blood vessels are tortuous and run zig-zag in many portions as they approach the tumor. There are in evidence many newly formed blood vessels in the tumor as well as in the vicinity of the tumor. The tortuous zig-zag course of the vessels are even shown by the ulnar and radial arteries as they branch off from the brachial artery. As in case III, the upper central portion is devoid of blood vessels. Again the vessels in the vicinity of the tumor are displaced from their original bed.

The enlarged X-ray (Fig. 13) at this stage of growth reveals the small net-work of blood vessels circumscribing the tumor. When compared to Case I (Fig. 5), there are numerous multiplications of newly formed vessels.

Case V. No. 7, white leghorn hen. A fragment of sarcoma was transplanted five weeks before under the right wing. The fowl was killed in the usual manner, and 2 cc of umbrathor was injected into the right brachio-cephalic artery and an X-ray taken.

The tumor was about the size of a hen's egg, having a necrosed area in the upper central portion with a cavity of 2 cm $\times$ $2\frac{1}{2}$ cm. The skin over the tumor was greenish-white; the consistency of the growth was soft. On section the medial aspect of the tumor was hemorrhagic. The portion adjacent to the muscle was firmly attached to it and harder than the rest. A portion of the muscle bed had shown infiltration.

The arteriogram (Fig. 14) shows a tumor 3 cm $\times$ $4\frac{1}{2}$ cm in diameter. It is covered with a thin net-work of blood vessels. The growth is so massive that the source of the nutritious vessels are not plainly visible. However, there are a few branches from the brachial artery approaching the tumor wall. The vessels, approaching the tumor, are small compared to the other figures of the smaller growth. Apparently at this stage of growth, the blood-vessels are decreased in number due to the expansive invasion of the malignant cells. However, still at the base of the tumor, there are seen considerable numbers of minute newly formed blood vessels. The upper central pole of the tumor, an area of $2\frac{1}{2}$ cm $\times$ 2 cm, is devoid of blood vessels. This is the necrotic area where the skin is destroyed. One thing is apparent at this stage to wit, that the marked multiplication and the zig-zagness of the vessels have disappeared. The radial and the ulnar arteries are not very plain. The opaque media have some difficulty entering them because of the external pressure of the tumor.

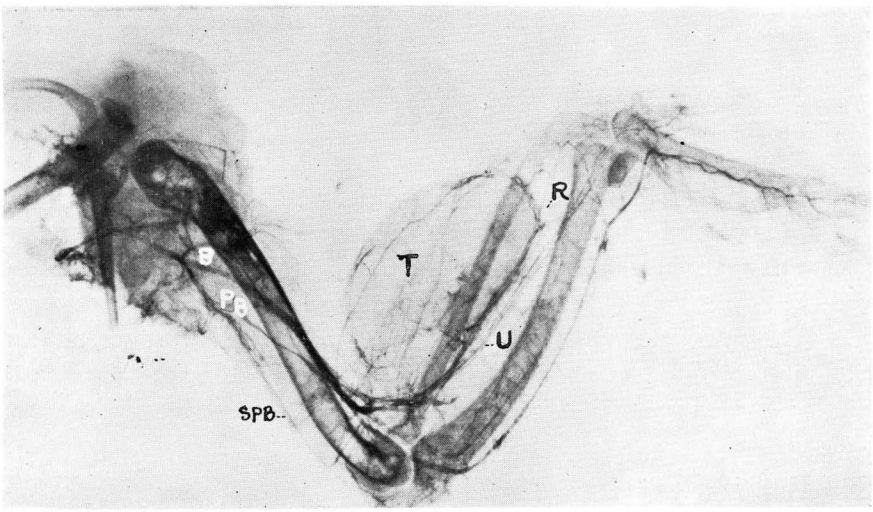

Fig. 14. Arteriogram of five week old sarcoma grown under the right wing. Note the scarceness of the blood vessels as compared to the previous figures. *T*, tumor; *B*, brachial artery; *P. B*, profunda brachial artery; *S. P. B*, superficial profunda brachial artery; *U*, ulnar artery; *R*, radial artery.

VIII. ARTERIOGRAPHY ON SARCOMA TRANSPLANTED INTO THE MUSCLE TISSUE OF THE FORELIMB

Case VI. No. 8, white leghorn hen. This hen was inoculated 24 days before with emulsion of sarcoma into the muscles lying between the radius and ulnar of both forelimbs. There was an oval diffuse mass, light bluish in color, about the size of a pigeon's egg. This mass in either forelimb was soft and fluctuating in consistency. On section later there were soft hemorrhagic areas in both wings where the opaque media had entered. These hemorrhagic areas were partly necrotic and filled with mucoid substance. Both tumor masses were well fixed in the muscles and showed expansive infiltration into the normal tissues. The bones were not involved.

The fowl was killed in the usual manner, and 2 cc of umbrathor was injected into the right and left brachio-cephalic arteries and X-ray taken. The arteriograms of both limbs reveal hazy areas of tumor mass occupying the muscular areas of ulnar and radius. The central portions are spotted with hemorrhagic areas in which the opaque media have entered. The peripheral portions are not densely surrounded with blood vessels as yet. The ulnar and radial arteries as well as

their branches are commencing to under go changes as to their position and size. The striking difference in this case, when compared to the former subcutaneous cases, is that the radiating net-work of blood vessels are not in evidence.

Case VII. No. 9, white leghorn hen. This hen was inoculated with emulsion of sarcoma 28 days before. The place of inoculation was the muscles of the left forelimb between the ulnar and the radius. As usual the hen was bled to death, and $2\frac{1}{2}$ cc of umbrathor was injected into the left brachio-cephalic and an X-ray taken.

The arteriogram (Fig. 15) reveals multiple blood vessels encircling the tumor. By their numbers, they are newly formed branches of both ulnar and radial arteries. In addition, the cutaneous branches join in to form a conglomerating net-work on the periphery of the tumor. The vessels are wavy in character and give the impression of being wildly distributed. The so called nutritional vessels are derived from both the ulnar and radial arteries. Even the distal portions of these main trunks of the forelimb give off branches, and turn backward, simulating recurrent vessels to assume the role of feeding the tumor with blood. All of these branches have undergone enlargement. The central portion is hemorrhagic and is revealed by the heavy shadow cast by the injecting media.

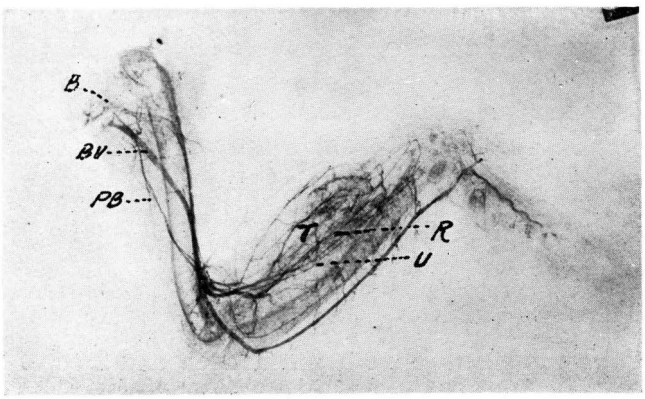

Fig. 15. Arteriogram of sarcoma transplanted into the muscles of the wing. Note the multiplication of blood vessels encircling the tumor. *T*, tumor; *B*, brachial artery; *BV*, brachial vein; *PB*, profunda brachial artery; *R*, radial artery; *U*, ulnar artery.

Upon section, it was found that the tumor had occupied the muscle area of the forelimb and diffusely enlarged to a size of a pigeon's egg.

The central portion was necrotic and filled with mucoid substance. The periphery was well infiltrated, showing traces of mucoid substance in the muscle tissue. There were abundant enlarged vessels encircling the tumor; the vessels were distributed in a disorderly way.

Case VIII. No. 16, white leghorn hen. This hen was inoculated with emulsion of sarcoma $4\frac{1}{2}$ weeks before into the muscles of the forelimb adjacent to the radius. Upon bleeding to death, $2\frac{1}{2}$ cc of umbrathor was injected into the left brachio-cephailc artery.

The arteriogram (Fig. 16). reveals that the tumor has apparently invaded into the subcutaneous tissue of the wing, extending outward, toward the anterior border of the wing. There is a massive hemorrhagic area along the muscles of the radius and the central portion of the tumor. The ulnar and the radial arteries are enlarged. The various branches given off from these main trunks are very irregular and are not plainly visible due to the extensive hemorrhagic areas. The brachial artery has given off two main nutritional branches to the tumor. The left border of the tumor presents a conglomerating network of blood vessels which are off shoots from the profunda brachial.

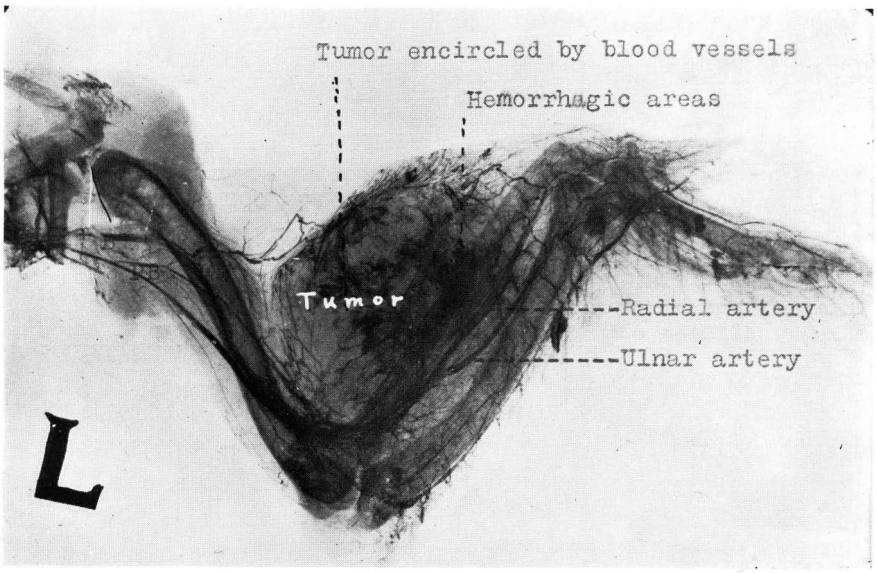

Fig. 16. Arteriogram of sarcoma transplanted into the muscles of the wing. Note the heavy shadow cast in the lower central portion due to hemorrhage. *B*, brachial artery; *PB*, profunda brachial artery; *R*, radial artery; *U*, ulnar artery.

The anterior border of the tumor presents fine radiating capillaries; the lighter ones are apparently veins. The right and lower borders of the tumor cast a heavy shadow due to hemorrhages. As usual the blood vessels, in all respects, are disorderly placed, and vascularity increased.

The above arteriographic picture was confirmed by the section of the tumor. The tumor was 4 cm × 4 cm, and the consistency was soft in the central and radial region. These soft areas were filled either with bloody or pure mucoid substance. The muscles around the radius were infiltrated with strands of malignant tissue.

IX. ARTERIOGRAPHY ON SARCOMA TRANSPLANTED INTO THE JOINTS OF THE FORELIMB

Case IX. No. 27, white leghorn hen. This hen was transplanted with emulsion of sarcoma into the left elbow joint 20 days before. In the usual manner, upon bleeding to death, $2\frac{1}{2}$ cc of umbrathor was injected into the left brachio-cephalic artery and an X-ray taken. The arteriogram shows opaque spots in the central portion of the tumor. The surrounding blood vessels are not very visible. However, at high magnification, there is a net-work of newly formed vessels especially circumscribing the left border of the tumor. The nutritional vessels that join to form the vascular net-work are the superficial branches of the profunda brachial and the superior collateral ulnaris. Neither the recurrent radial nor the ulnar artery is visible. The tumor mass has filled the joint cavity, and the articular surface is not in view. The proximal portions of the ulnar and radial arteries are shoved upward.

On section, the tumor was about the size of a finger tip; it was well fixed and attached to the capsule of the joint, as well as to the periosteum of the articular bones. It showed a small amount of mucoid substance.

Case X. No. 28, white leghorn hen. Likewise this hen was transplanted with sarcoma emulsion 20 days before into the right elbow joint. In the usual manner after the hen was bled to death, $2\frac{1}{2}$ cc of umbrathor was injected into the right elbow joint and an X-ray taken.

The arteriogram shows a circumscribed tumor, apparently receiving its blood supply from the profunda brachial artery, giveng branches mainly to the right side of the tumor. The left side presents in view the recurrent ulnar artery. The minute net-work of blood vessels

is not visible unless magnified. However, it is confused with the cutaneous blood vessels.

On section the tumor was about the size of a lima bean, filling the joint cavity, and has infiltrated backward along the humeral bone. It was well fixed to the periosteum; there was apparently no mucoid substance.

Case XI. No. 32, white leghorn hen. Sarcoma emulsion was transplanted into the elbow joint of the forelimb 28 days before. There was a growth about the size of a pigeon's egg. The lower pole of the tumor showed a small area of necrosis. The tumor consistency was soft; on section there was apparently no mucoid substance. It extended outward and mostly anteriorly from the joint.

The arteriogram (Fig. 17) shows the tumor surrounded by a network of blood vessels at its base; the superficial area is devoid of the ramifying vessels. The anterior border of the tumor is supplied with

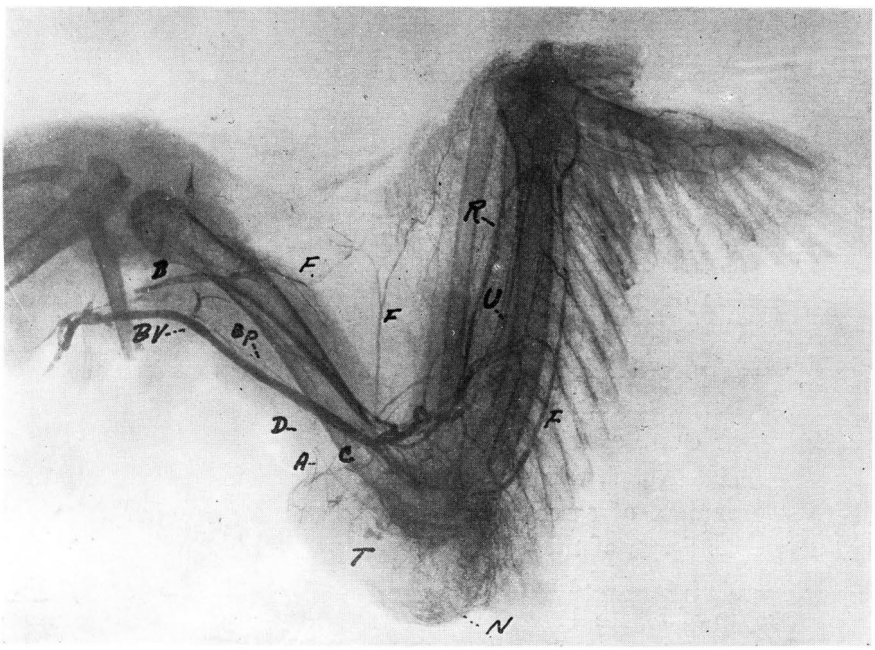

Fig. 17. Arteriogram of the elbow joint of the forelimb, transplanted with the sarcoma emulsion. *T*, tumor; *B*, brachial artery; *BV*, brachial vein; *BP*. brachial profunda artery; *A*, branch of superficial brachial profunda; *C*, superior collateral ulnaris artery; *R*, radial artery; *U*, ulnar artery; *F*, cutaneous branch; *N*, necrotic area.

the superficial branch of the profunda brachialis, which gives off two branches. Aside from this, the superior collateral ulnaris joins to feed the anterior portion of the tumor. Several branches from the ulnar artery, including the recurrent ulnaris, are shown to give branches to the tumor at the upper and the posterior aspect. The posterior aspect is a mass of ramifying vessels which can not be identified. The outline of the joint is missing. The ulnar and radial arteries at their bifurcation are shoved upward, and portion of the vessels are curled up. The vessels are all enlarged and lenghened. Due to obstruction, and probably pressure, the brachial vein is filled with the opaque media.

X. ARTERIOGRAPHY ON SARCOMA TRANSPLANTED INTO THE HINDLIMB

Case XII. No. 21, white leghorn hen; superficial tumor on calf of left leg. A fragment of sarcoma was transplanted 3 weeks before under the skin of the lower left leg. An irregular nodular mass was seen elevated above the skin. The center of the mass formed a crater caused by necrosis. The necrosis was evidenced by the black tissue in the crater itself. This growth was, however, well localized. In the usual manner, the leg was subjected to arteriography. The arteriogram shows a small amount of delicate vessels on the base of the tumor. They are apparently cutaneous branches, derived from the muscle branches of the femoral artery, and tibialis posticus artery. The superior portion of the tumor is circumscribed by enlarged vessels, which are newly formed vessels of the calf arteries that could be traced to the fomoral artery. The X-ray shows a crater, and its vicinity is entirely devoid of blood vessels. The scanty supply of blood to the tumor is no doubt the cause of the central necrosis.

Case XIII. No. 7, white leghorn hen; tumor on the calf of right leg. A fragment of sarcoma was transplanted 24 days before in the subcutaneous tissue of the calf of right leg. A tumor, elevated above the skin, was a size of a navy bean with a central necrosis of an area about the tip of a little finger. There was no infiltration in the muscle. The leg was subjected to arteriography with the injection of umbrathor in the usual manner.

The arteriogram (Fig. 18) shows that the primary nutritive vessels are derived from the cutaneous branch of the muscularis femoral, an

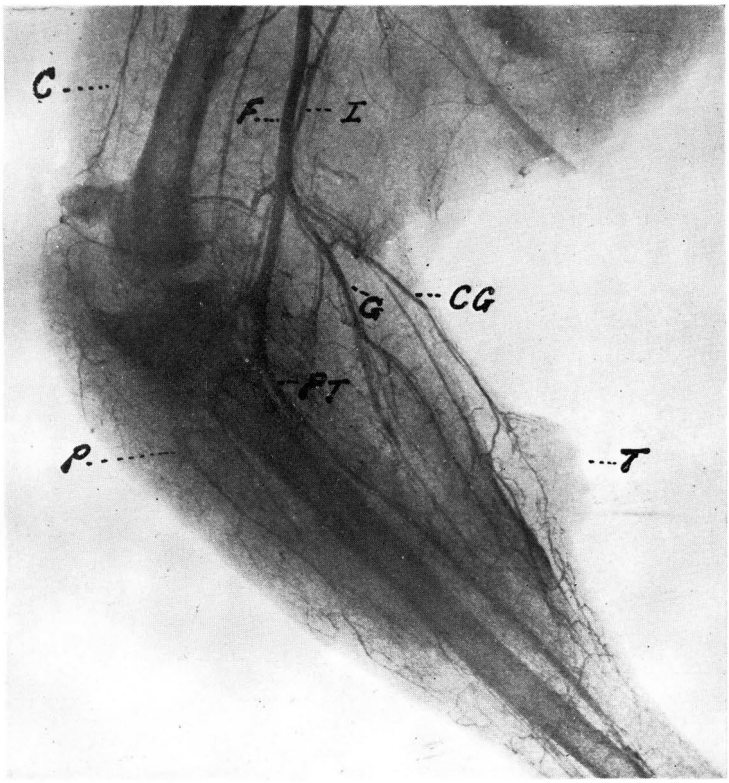

Fig. 18. Arteriogram of sarcoma grafted in the calf of the leg. Note the vessels entering the tumor, *T*, tumor; *I*, ishiadic artery; *C*, crural artery: *F*, femoral vein; *G*, gastrocnemius artery; *CG*, cutaneous branch of gastrocnemius artery; *PT*, posterior tibial artery; *P*, peroneal artery.

offshoot of the femoral. The vessel could be traced toward the source. From the upper pole of the tumor, the nutritive vessel enters and encircles the middle one-half of the tumor. The inner one-half of the tumor is enveloped by a delicate net-work of vessels. The vessels adjacent to the tumor are tortuous, and more or less enlarged.

The enlarged view (Fig. 19) of the above arteriogram, shows a network of ramifying blood vessels, approaching the tumor from both ends, which finally unite in the central portion of the tumor. The neighboring vessels of the tumor are markedly enlarged, lenghened, and twisted in all manners. As usual, the area of the crater is devoid of blood vessels.

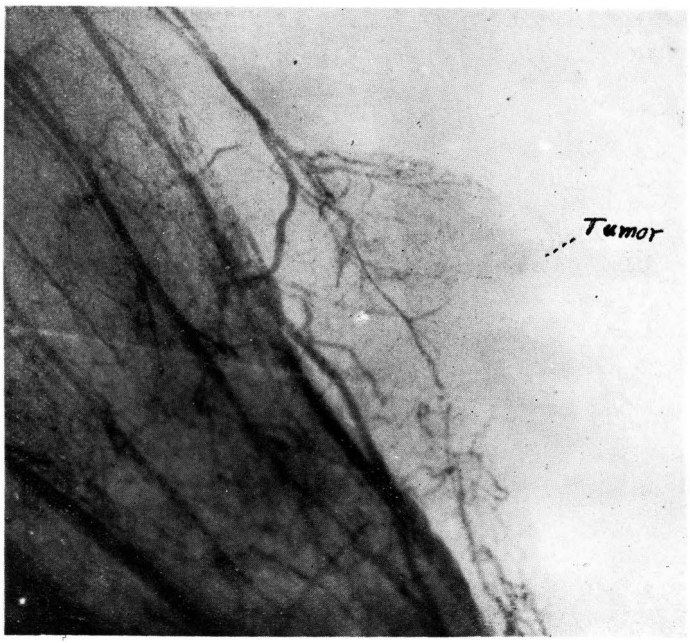

Fig. 19. Enlarged view of figure 18. Note the vessels encircling the base of the tumor.

Case XIV. No. 11, white leghorn hen. A sarcoma fragment was transplanted into the right calf, one month and eleven days before. There was on the lateral surface of the thigh and calf a tumor about the size of a hen's egg. It was elevated somewhat above the skin, soft in consistency, and showed a chocolate mucoid mass in the center. In the usual manner the leg was subjected to arteriography.

The arteriogram reveals a tumor mass, 4 cm $\times$ 5 cm. The femoral artery and its branches are the main source of the blood supply to the tumor. These vessels are markedly enlarged, and at the base of the tumor, are massive ramifications of vessels, which are branches mainly of femoral, before it bifurcates into the anterior and posterior tibial arteries. Due to pressure the posterior tibial is diminished in size. The central portion of the tumor is devoid of blood vessels.

XI. COMMENTS ON ARTERIOGRAM OF SUBCUTANEOUS, INTRAMUSCULAR, AND INTRA-ARTICULAR TRANSPLANTED SARCOMA

Under different headings, I have described arteriography of tumors, grafted subcutaneously, intra-muscularly, and intra-articularly by using

the hind forelimbs of the fowl. For the subcutaneous grafting, a fragment of tissue was embedded under the skin; for intra-muscular and intra-articular, the sarcoma emulsion was injected to produce growths. Different stages of the growth have been subjected to the arteriography. As a whole, these two methods of transplantation have given similar results, but the former method is considered better in observing the development of the newly formed vessels.

Among the five cases, grafted under the skin of the wing, the malignancy of the growth can be readily recognized. Although there are slight variations in the speed of growth and in the development of newly formed vessels, essentially the vascular picture is the same. The characteristic vascular picture of malignant growth; viz., enlargement of vessels, lengthening of vessels, multiplication of vessels, displacement and twisting vessels, and entire disappearance of vessels in the later stage, etc. are found in the different stages of the growth. The above conditions are much more in evidence in cases of subcutaneous or intra-muscular growths.

First let us see what changes have occurred in the main trunk arteries. In glancing over the 14 cases, although not in evidence in the early stages, the main trunk arteries are without question enlarged in response to the increased flow of blood. Such conditions are represented by the brachial arteries of Figs. 9, 12 and 17. However, as the tumor enlarges to a certain extent, the trunk arteries begin to show decrease in size, such as in the radial artery of figures 12 and 14. The term increase or decrease is a relative term, and accurate measurements are impossible, due to the smallness of the vessels seen on the arteriogram.

NOTHNAGEL(117) experimented by tying the main artery of the extremity, and found there was enlagement in the caliber of the collateral arteries. On this basis, according to SAITO(116) and his colleagues, it is reasonable to think if a tumor is produced and circulation increased, there is good reason to believe that the main artery and the nutritional arteries of the tumor would increase in size. In working with human cases, SAITO(17) found enlargement of the trunk arteries in tumors. This finding was obtained by comparing with the normal side.

In addition to the increase of decrease in sizes of the trunk arteries, there are changes caused by the pressure of the tumor, which are tortuosity, obliteration, and displacement from the beds. Such

characteristics have been noticed in these cases, and typical examples are figures 12 and 13, where the vessels are irregularly distributed and highly curved.

GOLDMANN(118) mentioned the fact that the abnormal, irregular distribution of the blood vessels is present. He stated that such changes are characteristics in the malignant tumors, but also in cases of tuberculosis as well as in syphilis. He concluded that as long as there was the power of the tissue to grow, in any case of injury as a reaction, there was a possibility for the blood vessels to multiply.

It is easy to understand why the displacement of the vessels takes place. Since the fowl sarcoma expands by proliferation, the nearby vessels are necessarily shoved away. Finally the trunk blood vessel may be obliterated. However, in my series of cases, there are none of that type. According to ASAI(108), in his rabbit's lung sarcoma experiment, he has seen the tumor cells block or thrombose the pulmonary artery, which he has recognized by the X-ray, and at necropsy.

As to the lengthening of the vessels, I may say that it always goes hand in hand with the enlargement. It is a known fact that vessels prolong as well as enlarge in their caliber. However, normally due to the lengthening of the distal end where the vessel runs, there is no tortuosity or unnecessary twisting. The twisted or wavy course of the vessels, as they approach the tumor, is one of the characteristics of neoplastic vessels.

The multiplication of blood vessels is one of the outstanding characteristics of sarcoma and other malignant tumors. The exact cause of the increase, whether nervous, or local hormone, is still unsettled by both advocates. The increase in vessels is confirmed especially with the subcutaneous and intra-muscular growths. The joint and the leg tumors, however, have not shown much increase in the blood vessels. For the leg, necrosis has accounted for the decrease; and for the joint, the enclosed condition within a joint capsule probably has accounted for the scantiness of the vessels. The scantiness of vessels may not mean non-malignancy. Occasionally there are retarded growths, sometime found in rabbits' sarcoma with central necrosis, of which I have experimented in my research on "malignant joint absorption". In scirrhus cancer, one may find less amount of blood vessels.

The newly formed vessels are seen to envelope the tumor by

forming a fine network. The malignant tumors, according to TANAKA (100) in his studies on blood vessels by microscopic section, grow rapidly and without limit; and thus they multiply to form an independent system from that of the host. The network of vessels are shown very definitely in the rapidly growing tumors, and the contrary is true with the cases with crater necrosis (Figs. 9 & 10).

According to RIBBERT(98), (99), he has recognized in the malignant tumors, thin walled vessels like the capillaries. These vessels have thin walls, but more or less a large lumina. He has also recognized in the wall of these vessels malignant tumor cells; the vessels grow as the tumor grows. W. E. GYE and W. J. PURDY(119) also mentioned, "that frequently the neoplastic tissue could be seen to have penetrated the vessel wall and sometimes to have extended into the lumen". I have seen in my microscopic sections (Figs. 7 & 8) RIBBERT's so called capillary vessels. SAITO(17) has confirmed RIBBERT's finding in human cases. RIBBERT(98), by microscopic section, has recognized a regular formed vessel in the tumor with proper wall. According to SAITO(17), this is the primary nutritive vessel seen in the arteriogram of tumor.

If we follow our predecessors in calling the vessels which feed the tumor as primary, secondary, or tertiary nutritive vessels, the fowl sarcoma is not exempt from this nomenclature. The term primary nutritive vessel denotes the one which supplies the tumor first. By the enlargement of the tumor, the distal artery may join in to form the secondary or tertiary nutritional vessels. This is well shown in tumors grown under the skin (Figs. 5, 9 & 12). The term nutrient artery of the tumor is first used by J. A. SAMPSON(102) in describing the arteries of uterine myoma. In the fowl sarcoma also, the nutrient arteries are enlarged, prolonged, branched out, and zig-zagged.

Aside from the nutrient arteries, the vessels nearby in lesser degree, have the above characteristics. This is not only true in fowl sarcoma, but also true in human cases. The marked bleeding during operation of human sarcoma from the surrounding tissue is nothing new.

The best method of transplanting tumor, as mentioned before, in order to get a good arteriogram is by fragment embedding. This method is feasible in subcutaneous location where incision and suture is quite possible. However, in deeply situated muscles due to the possible injury to the vessels as well as the overlying tissue, injection of emulsion of sarcoma is chosen as a necessity. The three cases VI,

VII, and VIII are typical muscular inoculated examples. They grow rather diffusely without definite circumscribed border. The expansive proliferation of the tumor is manifested by its invasion and destruction into the intermuscular stroma, and into the subcutaneous tissue of lesser resistance.

In the early stage as in Case VI, the blood vessels are not of a massive type. However, in the later stage, as in Case VII, multiplication of blood vessels shows itself. Still later, as in Case VIII, because of necrosis and hemorrhage, the outline of the vessels becomes indistinct, and marked extravasation of the opaque media gives a picture of sinus within the tumor substance. The disturbance of the blood vessels has caused multiple necrosis in the tumor. TANAKA(100), in his study of malignant blood vessels, states that in fast growing tumors, the malignant cells penetrate into the blood vessel wall and obstruct, thereby causing nutritive disturbance. GYE and PURDY(119) note that, "the tumors are found to be highly vascular and are usually hemorrhagic; often there is a central cavity filled with blood—there is usually a quantity of viscous slimy fluid admixed with the bloody tumor tissue." I have found the above description to be true in my cases, and the arteriogram has confirmed this.

SAITO and SAKURAI(120) in their studies on arteriography of uterus, before and after pregnancy, have seen masses of heavy shadow in the ovary which is noted to be ruptured graffian follicle. This somewhat resembles that of the hemorrhagic areas of fowl sarcoma.

Inplantation of sarcoma emulsion into joint cavity invariably produces growth, but when compared to subcutaneous or intra-muscular inoculation, it is slow to grow. Probably this is due to the enclosed condition of the joint cavity, in which it is surrounded with a rather tough capsular ligament. Another striking thing about joint sarcoma is that little or no mucoid substance is present compared to that of the subcutaneous and intramuscular growth.

The arteriogram does not reveal any too good a network of vessels when compared to that of the subcutaneous growth. This is probably due to the tumor being deeply placed and enclosed within a capsule. The nutritional vessels are the ones in close proximity to the joint, such as the recurrent ulnar and radial, the superior ulnar and radial collateral, and profunda brachialis. The characteristic vessel picture of malignant tumor, such as enlargement and tortuousness, is present.

The arteriograms of the leg tumors, generally speaking, have not shown many blood vessel changes. As stated before this may be accounted for by the necrotic character of the tumor. However, those present with blood vessels are typical of malignancy. HASEGAWA(121), in his experiment with rabbit's sarcoma has noticed also devoid areas of blood vessels in necrotic tumors. He has experimented on the gangrene formation in the mesentery by tying the blood vessels, and noted that the necrosis first started in the distal artery. It is true also in the fowl sarcoma; necrosis occurs first in the area of the distal artery (Fig. 14).

The difference in cases of other arteriograms, such as in rabbits' sarcoma (HASEGAWA, KANNO, ASAI, CHIN) is that, due to mocoid and hemorrhagic extravasation, the blood vessel pictures are often times interfered with by massive spots. In the early stage of growth, it may simulate that of the rabbit's sarcoma or that of the human sarcoma. However, at the later stage, when the tumor is soft and characterized by a large amount of mucoid substance associated with hemorrhage, there are massive spots. In such cases the nutritional vessels of the tumor are difficult to identify (Figs. 14 & 16).

XII. ARTERIOGRAPHY ON SARCOMA TRANSPLANTED INTO INTESTINE

Case XV. No. 23, white leghorn hen. This hen was operated on 18 days before. At that time the abdomen was opened, and after bringing the intestine into view, 0·1 cc of sarcoma emulsion was injected into the serosa. The abdominal incision was closed with suture. Then the hen was killed as usual by severing the carotid artery. Upon exposing the descending aorta, 10 cc of cinnabar preparation was injected into it. After the solidification of the injected material, the intestine was dissected out per se. with the blood vessels attached to it. There was a small growth about the size of a rice grain elevated above the serosa. It was greyish white, and showed blood vessel leading to its base. Only a small network of blood vessels was seen at the base of the tumor. The consistency of the tumor was soft. A loop of intestine, about 3 inches in length, was severed, and intestinal content was washed out. Then the loop was inflated with air, to permit a distinct picture of the wall by the X-ray.

The arteriogram (Fig. 20) shows the tumor occupying a small portion of the intestinal wall. The vicinity of the intestinal wall is

slightly thickened. There are two main sources of nutrition. The two endarteries encircling the intestine anteriorly and posteriorly join in to supply the required blood to the tumor. These two arteries are apparently not enlarged compared to the endarteries of the normal

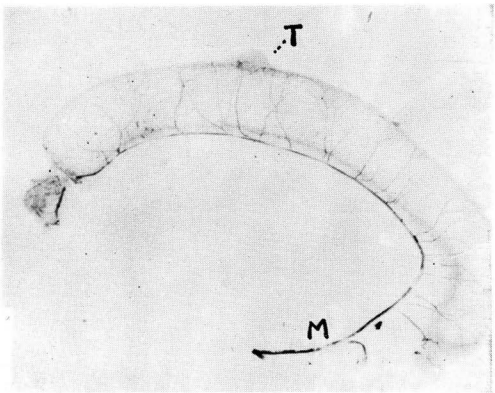

Fig. 20. Arteriogram of portion of intestine showing sarcoma nodule. *T*, tumor; *M*, mesenteric vessel.

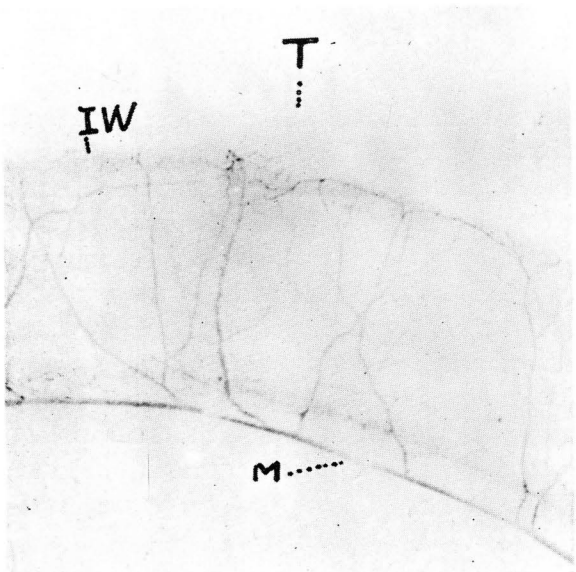

Fig. 21. Enlarged view of figure 20. *T*, tumor; *M*, mesenteric vessel; *IW*, intestinal wall.

region. They join in at the base of the tumor to produce a scanty network of vessels, which are difficult to identify unless well magnified (Fig. 21).

Case XVI. No. 28, white leghorn hen. This hen was injected with emulsion of sarcoma into the brachial vein of the right forelimb. After a month, the hen was killed and found to have tumor growths in the stomach, and the wall of the large bowel. Ten cubic centimeters of cinnabar were injected into the descending aorta; the large bowel was dissected out and X-ray taken.

Two tumors were located slightly above the rectum. The lower was about the size of a pea and the upper about the size of a rice grain. They were in close proximity and were elevated above the serosa of the gut wall. On section, the tumor had involved only the serous coat; there was no involvement either in the muscular or the mucous coat (Fig. 22).

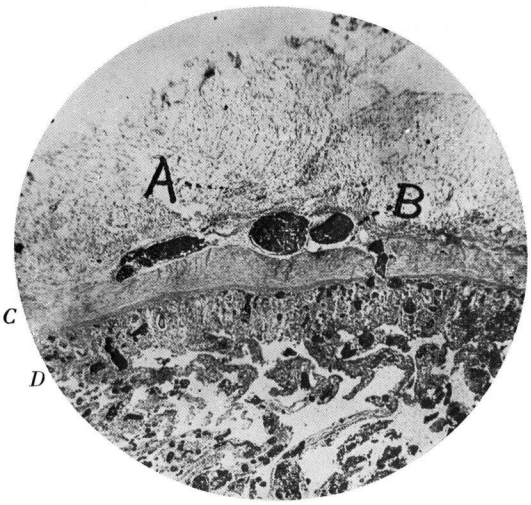

Fig. 22. Photomicrograph of sarcoma of intestinal wall. *A*, sarcoma cells; *B*, blood vessels; *C*, muscle coat; *D*, mucosa. Noto the engorged blood vessels under the tumor.

The arteriogram (Fig. 23) presents a wavy outline of blood vessels at the base in both cases. These vessels are the two lowermost branches of the inferior hemorrhoidal artery. It is hard to make out whether the above mentioned vessels are enlarged or not, even under the enlarged view. The elevated portions of the tumors are devoid

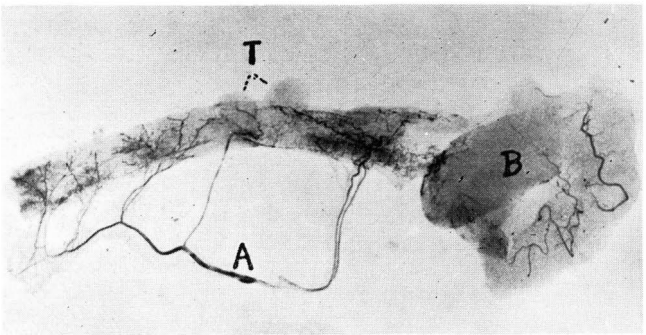

Fig. 23. Arteriogram of the large intestine near the rectum showing two small sarcomata on the wall. *T*, tumor; *A*, inferior hemorrhoidal artery; *B*. rectum.

of blood vessels. Apparently they obtain their blood supply from the base of the tumor.

The enlarged view (Fig. 24) shows many blood vessels in the vicinity of the tumor, which are markedly more zig-zag in contour than that of Case XV, Fig. 27.

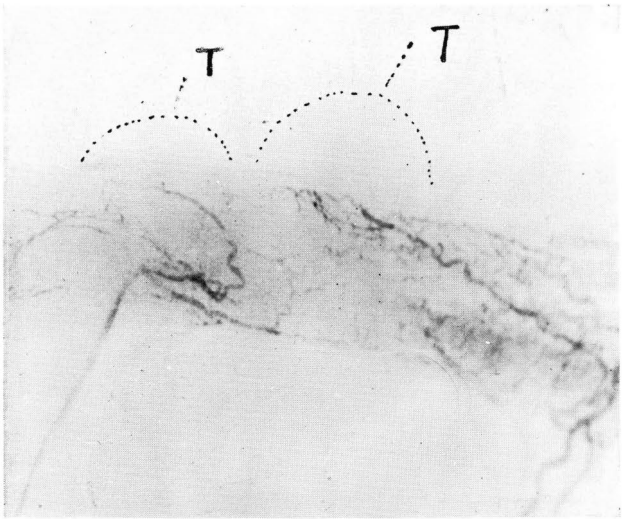

Fig. 24. Enlarged view of figure 29. *T*, tumor. Note the wavy vessels.

Case XVII. No. 37, white leghorn hen. Sarcoma emulsion was injected into the abdominal cavity one month before. Upon death of the hen, about 6 cc of umbrathor was injected into the celiac axis, and an X-ray taken.

There was a massive greyish tumor on the duodenum, especially on the two ends of the loop. The growth had circumscribed the gut, and also the pancreas. Macroscopically there was no increase in blood vessels. On the contrary, the tumor area had scanty blood vesels.

The ateriogram (Fig. 25) shows that the portion covered with the tumor is devoid of blood vessels. The duodenal artery and its branches of intestinal arteries have decreased in size, and those still remaining are abruptly cut short, as soon as they approach the intestinal wall. The usual characteristics of the tumor vessels are missing, probably due to the obstruction.

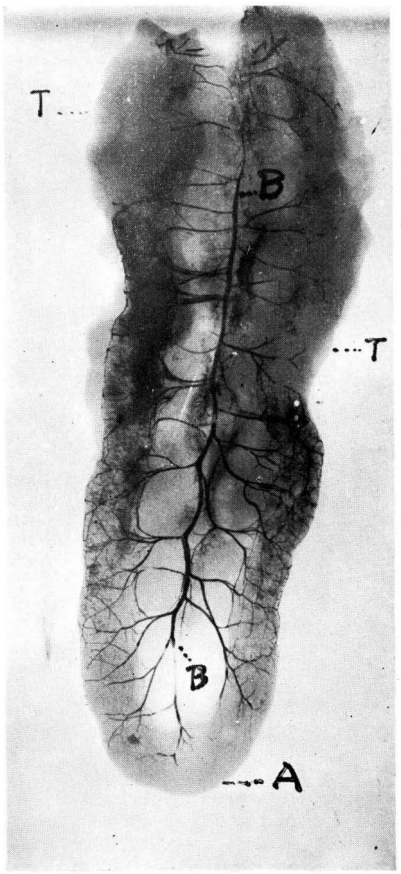

Fig. 25. Arteriogram of sarcoma on the duodenum. Note the absence of vessels. *T*, tumor; *A*, duodenal loop; *B*, duodenal artery,

XIII. ARTERIOGRAPHY OF MESENTERIC AND PERITONEAL SARCOMA

Case XVIII. No. 39, white leghorn hen. This hen was inoculated with the emulsion of sarcoma, one month before into the stomach wall. The fowl was killed in the usual manner. There was a large growth on the anterior wall of the stomach, about the size of a closed fist. In addition there was a flat soft growth, about 4 cm × 5 cm on the mesentery (Fig. 26 *A*). Likewise on the mesentery, there was a slightly elevated soft growth, 2 cm × 3 cm adjacent to the intestine (Fig. 26 *B*). Both of these tumors were greyish white and soft in consistency; the blood vessels were scanty and not enlarged. The microscopic section of tumor (*B*) revealed a massive area of spindle cells and scanty amount of blood vessels.

The arteriogram (Fig. 26) shows both tumors are embedded in the mesentery. The mesenteric artery gives off two branches at the base of the large tumor. These two vessels, in turn, distribute twelve to thirteen short branches to the tumor. There is no ramification of vessels as we find in subcutaneous tumors.

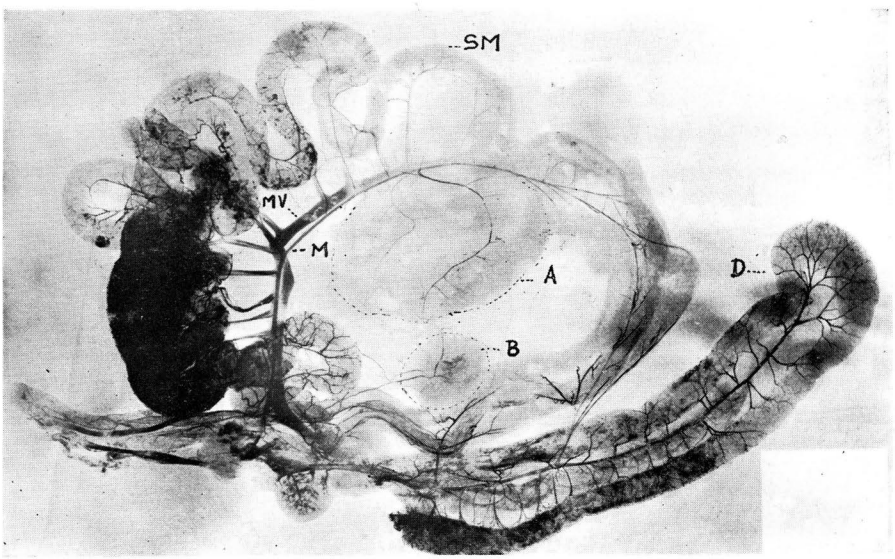

Fig. 26. Arteriogram of the mesentery sarcoma. *A*, large flat tumor; *B*, nodular tumor; *M*, mesenteric artery; *SM*, small intestine; *D*, duodenum; *MV*, mesenteric vein.

To the second small tumor adjacent to the intestine, a branch vessel is given off from the mesenteric artery about at its base. The terminal end of this vessel is divided into two branches in the midst of the tumor. One branch assumes a delicate network on the surface. However, the notwork as seen in the enlarged view (Fig. 27), occupies only a very small portion of the tumor. Again as in the former, the blood vessels are scanty in comparison with the tumors grafted under the skin. The vessels are so delicate that it is very difficult to determine, whether the lumina have enlarged or not.

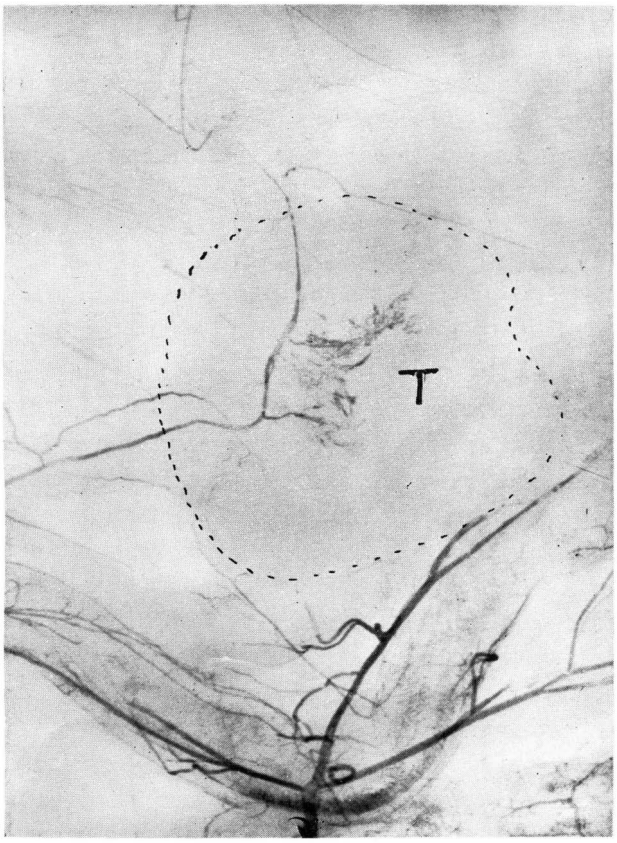

Fig. 27. Enlarged view of Fig. 32. *B. T*, tumor.

Case XIX. No. 29, white leghorn hen. Sarcoma emulsion was inoculated into the abdominal cavity one month before. Upon killing

the fowl, a flat tumor was found attached to the serosa of the gallbladder with its blood vessels branching out from the cystic artery. There were also two pea-sized metastases on the surface of the liver. In the usual manner, sufficient amount of cinnabar was injected into the descending aorta and arteriogram obtained. The flat tumor was about, 3 cm × 4 cm, and more or less triangular in shape. Like the mesenteric tumor, it was soft, glistening, greyish white, and contained mucoid substance, especially around the gall-bladder.

The arteriogram of the liver is normal; the metastatic tumors were superficially placed on the surface so that no change had developed in the liver substance.

The arteriogram of the tumor is characterized by a central blood vessel, apparently a newly formed one, branching off from the cystic artery of the gall-bladder, and at its termination it has given off ramification of delicate vessels to supply blood to the growing portion of the tumor. As in the former Case XVIII, figure 26, the blood vessel network is scanty.

XIV. ARTERIOGRAPHY ON SARCOMA TRANSPLANTED INTO STOMACH

Case XX. No. 33, white leghorn hen. Sarcoma emulsion was injected into the anterior wall of the stomach one month before. The fowl became very weak at the time of the experiment. There was a large growth about the size of a hen's egg on the lower anterior surface of the gizzard. It was greyish-white, soft, and very friable. The surface was very scanty with blood vessels. In the usual manner the arteriogram of the stomach was taken by injecting sufficient amount of cinnabar into the celiac axis. The content of the stomach was removed before the X-ray was taken.

At one glance at the X-ray (Fig. 28), it is very striking that the blood vessels are markedly reduced in size. The two branches of the stomach vessels have been transformed into the nutritional vessels of the tumor, entering from the superior pole. They give off irregular delicate vessels which penetrate into the tumor substance. Most of the large sized blood vessels end abruptly in the tumor. The finer network of vessels is lacking as might be seen in subcutaneous tumors. On the right upper corner of the tumor there are hemorrhagic areas, which are represented by heavy shadows. The lower peripheral portion of the tumor is devoid of blood vessels.

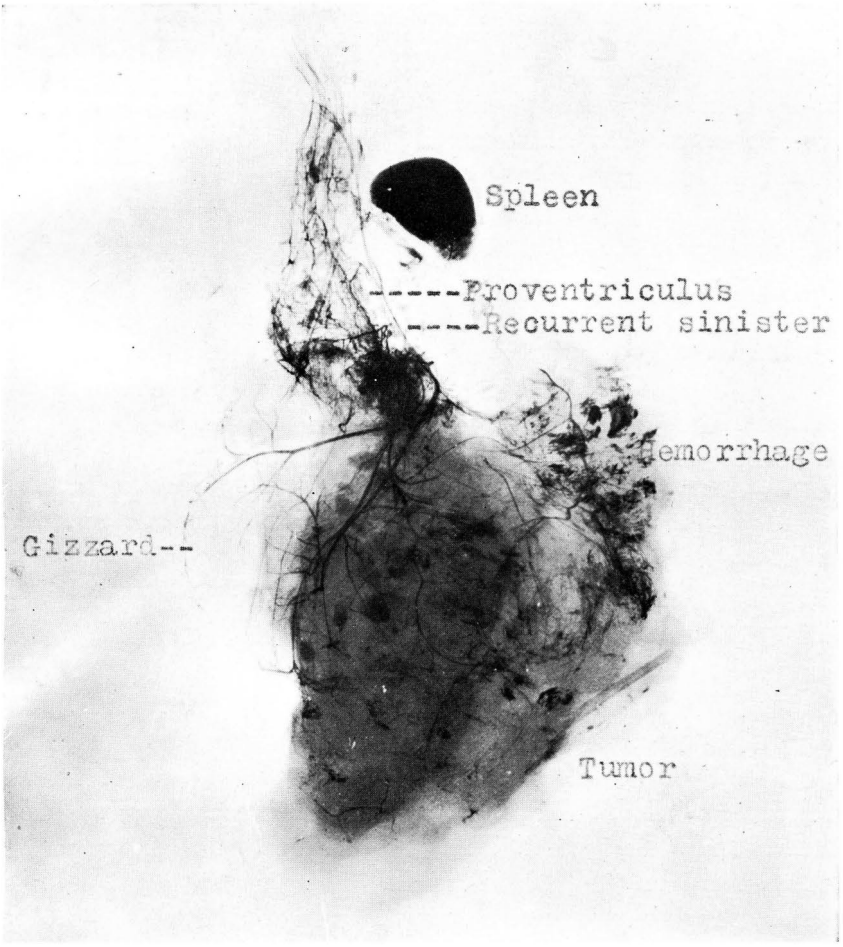

Fig. 28. Arteriogram of the stomach sarcoma.

On section there is a marked infiltration of tumor tissue into the muscle tissue. The mucosa is still not invaded.

Case XXI. No. 39, white leghorn hen. Sarcoma emulsion was inoculated one month before. There was a tremendous hemorrhagic growth on the anterior wall about the size of a closed fist. In addition, there was also a tumor about the size of a hen's egg on the posterior wall. These two tumors were so large that only a small upper portion of the gizzard was visible. The postero-lateral aspect of the tumor was

raw red, friable and covered with mucin. The anterior surface was covered with a thin gelatinous membrane.

The X-ray reveals a mass of grain-like shadow in the lower one-half of the gizzard which is cast by its content. The recurrent sinister artery which supplies the stomach is not very distinct. However, it passes down along the proventriculus and gives off innumerable branches to the gizzard; these branches are very markedly curled up. As the gizzard branch from the recurrrent sinister reaches the area of the tumor, it gives off multiple branches which are twisted, and two or three of them delicately run into the midst of the tumor. These vessels are very slender. The lower one-half of the tumor is devoid of blood vessels. In the right upper one-third of the tumor in which hemorrhagic areas are present, there is a massive shadow caused by the extravasation of the opaque substance.

Case XXII. No. 38, white leghorn hen. Sarcoma emulsions were injected into the vein of the forelimb one month before. It had caused a walnut sized tumor, adjacent to the upper portion of the gizzard; and a duck's egg sized tumor on the lower portion. Due to the heavy shadow cast by the stomach content, it is impossible to describe the blood vessels of the latter tumor. The former walnut-sized tumor is a growth from the wall of the stomach, adjacent to the pylorus. The recurrent sinister artery passes posterior to this tumor, and no branches are supplied to it. The source of blood supply to the growth is from the delicate network of blood vessels from the stomach wall. These vessels are very tiny and difficult to distinguish. The major portion of the tumor is devoid of blood vessels. Again, hemorrhagic areas are present through out the tumor as in the former cases.

XV. COMMENTS ON ARTERIOGRAM OF SARCOMA TRANSPLANTED INTO INTESTINE, MESENTERY AND STOMACH

The first two cases in section XII are growths on the intestinal wall, produced by injections of sarcoma emulsion into the wall. The sarcoma of the remaining cases of section XII and XIII are caused by injections into the abdominal cavity. It is noticeable that the growths are retarded in all abdominal cases. I find the optimum site of growth in the mesentery, is the area adjacent to the intestine, where the vessels are given off by the arch arteries of the mesentery. The cell emulsions come direclty into contact with the endothelial lining of the mesentery

or the peritoneum, and at length obtain a blood supply from the underlying tissue. OSHIMA(122) and others found the duodenum to be the site of much frequent growth in the abdominal cavity.

The arteriograms of both intestine and mesentery show only a scanty amount of blood vessels. The characteristic network as seen in the subcutaneous cases are lacking. The vessels, if present, are located at the base of the tumor. However, the enveloping blood vessels are not numerous, which may account for the slow growth. As usual in large tumors, the surrounding blood vessels disappear as in Case XVII, where the tumor covers the duodenum. In the early stage, there are newly formed blood vessels, acting as the nutritive vessels. The twisted character of the vessels are in evidence along the intestinal wall (Fig. 24). In the mesenteric tumor, the primary tumor nourishing artery is enlarged practically to the size of the trunk vessel. However, the delicate terminal network is hard to be recognized (Fig. 26 *A*).

KANNO(107) transplanted sarcoma into the rabbit's intestine and observed the vascular picture. He reported that new branch vessels were formed, and the trunk artery was enlarged. As to the mesentery, he stated, "In locations where no blood vessels can be seen macroscopically, Roentgen ray reveals the images of minute arteries on the mesentery which terminate at the position of the sarcoma. These are the nutritive arteries grown under the influences of the stimulus from the invisible, but physiologically functional blood vessels." If the above explanation is true, figure 26, can be said to resemble his case.

In summarizing, it is safe to say from my fow cases of sarcoma transplanted into the mesentery and intestine, that the vessels existing at the grafted position, undergo several changes as to size and number. Due to the smallness of the tumor, the development or modification of the vessels into nutritive arteries of different grades is not demonstrable in any of the cases, except in one (Case XVIII, Fig. 26).

As to the tumors of the stomach, I have succeeded in obtaining large growths. When compared to the intestinal growth, they are much larger. This is accounted for by the fact that the stomach is a much larger organ; its muscle seems to be an ideal site for the growth. KONUMA(123) found most of the metastases on the greater curvature (pars intermedius). They were mostly in the muscle tissue, and he gave the reason that the blood vessel to the stomach gives off two

branches, the right and left, which enter the pars intermedius through the serosa and into the muscle layer to produce a network. The site of the network is where the blood is stagnant, an optimum place for the metastases to lodge.

Despite the large growths obtained in my stomach cases, the blood vessels are small, delicate, and the characteristic tortuosity is missing; the newly formed vessels are present. KANNO(107) in rabbit's sarcoma transplanted into the gastric wall, found much tortuosity, enlarged vessels, etc. In my experience with fowl sarcoma on the gastric wall, I find this is not always true. The multiplication of blood vessels is in evidence, but they are so delicate that there are no means of measuring their caliber. Probably the softness and hemorrhagic characteristics of fowl sarcoma have rendered difficult the arteriography.

In general, the arteriograms of the gastric wall sarcoma reveal a modification of nutritive arteries to feed the tumor at its base, and hemorrhagic areas are much more common.

XVI. SUMMARY

1. The blood-vessel studies of fowl sarcoma by X-ray are made on twenty-two white leghorn hens.

2. Fragments or emulsions of the tumor tissue are transplanted into various tissues; viz., subcutaneous tissue, muscle, joint, mesentery, intestine, and stomach.

3. At first much difficulty has been encountered to obtain good X-rays because of the softness of the tumors which are invariably hemorrhagic.

4. Good growths have been obtained in the majority of the cases. The experimental fowls have been killed at various stages of the growth and a sufficient amount of opaque substances, either umbrathor or cinnabar has been injected into the desired arteries; X-ray pictures are taken after dissecting out the desired organs.

5. The blood vessels of the subcutaneous tumor are best seen to develop into the nutritional vessels; the other organs invariably show the same, but not so marked as that of the above.

6. Because of the hemorrhagic nature of the fowl sarcoma, there are heavy shadows cast in the midst of the tumor.

7. There is marked increase of blood vessels during the early stage

of the tumor; in the later stage the vessels are decreased, caused by obliteration and necrosis.

8. The so-called primary, secondary, tertiary blood vessels are formed as in the rabbit's sarcoma. However, those tumors, aside from the subcutaneous, and intramuscular growths, are rather difficult to demonstrate them.

9. The surrounding blood vessels of the tumor are newly formed and tortuous in their course.

10. The neighboring blood vessels are enlarged, lengthened, and displaced in different degrees; in certain instances the trunk vessels are enlarged.

11. In general the arteriographic pictures resemble those of the rabbits' sarcoma.

In closing the author wishes to express his sincere thanks and appreciation to Prof. Dr. MAKOTO SAITO for the assistance and suggestions which he has received. The author wishes also to make grateful acknowledgment to the staff of the Roentgen department for their coutesy in permitting the use of X-ray

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