SARCOMAS IN HAMSTERS AFTER INJECTION WITH ROUS CHICKEN TUMOR MATERIAL*

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It is generally believed that Rous sarcoma virus cannot attack mammalian cells and that it cannot induce sarcoma in mammals. The sarcoma can, however, under certain conditions be transplanted from chickens to mammals,—to immunologically shielded sites: brain (1, 6, 10), anterior chamber of the eye (16), diffusion chamber (5), or to blockaded (14) or cortisone-treated animals (12, 13, 17); but it will survive only for a short period. Kuwata (12, 13) transplanted the Rous sarcoma and its variant strain, 14(d)7, to hamsters. While the tumor always regressed within 10 days in untreated hamsters, it survived and grew for about 3 weeks in cortisone-treated animals. The hamster-grown tumors could be successfully transplanted to conditioned hamsters when the inoculum also contained an admixture of normal chick embryonic tissue. In addition, extracts of hamster-grown tumors could transform normal chick embryo tissue into sarcoma in conditioned hamsters. On the other hand, hamster embryo cells could not be transformed into sarcoma cells by chicken sarcoma extracts.

In the present investigation a virus was used, which is probably a peculiar variant (mutant) of the Rous sarcoma virus. A chicken carrying the growth was obtained from Dr. Schmidt-Ruppin,[1] and it has since been maintained in them by transplantation. It will be called the Schmidt-Ruppin strain. The virus can induce sarcoma in rats, mice, and guinea pigs with a high percentage of takes and it can produce sarcoma-like, though regressive, lesions in rabbits (3, 4, 7). In addition to these changes rats and rabbits develop multiple lymphogenous cysts. The results obtained in hamsters differ considerably from those obtained by Kuwata with his Rous sarcoma and its variant strain, 14(d)7.

Material and Methods

The Schmidt-Ruppin strain of sarcoma has previously been used by Ising-Iversen (11) and by Ahlström and Jonsson (3).

In our laboratory the sarcoma has been maintained in 2- to 3-week old White Leghorn chickens. They were implanted at roughly 14-day intervals to their breast muscle with 0.25 to 0.50 ml of finely minced sarcoma tissue, suspended in Hanks' solution containing 100 units

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[1] Dr. H. Schmidt-Ruppin, Frankfurt/Main-Hoechst, who has reported (personal communication) that he received it from the late Professor Ch. Oberling, Paris, and that it has since been serially transmitted in chickens in the conventional way.

of penicillin, 0.10 mg of streptomycin and 0.10 mg of chloromycetin per ml. After 1 to 2 weeks a bulky, greyish-white, partly viscous tumor with the usual gross and microscopical appearance of a Rous sarcoma develops at the site of inoculation. Implanted chickens were always kept in separate cages.

An extract of homogenized chicken sarcoma was prepared in the following way:—

Parts of the tumor were thoroughly ground with sterile glass powder for 15 minutes in a mortar and suspended 1:5 in Hanks' solution containing the antibiotics. The suspension was clarified in a MSE super multex centrifuge for 15 minutes at 4,000 RPM. The supernatant fluid was then taken off and centrifuged at 13,800 RPM (10,000 *g*) for 30 minutes at −3° in an International cold centrifuge. The new supernatant fluid was then cautiously removed and recentrifuged for the same time and at the same speed. The procedure was repeated yet again, and the final supernatant thus obtained was used for the experiments.

Syrian Golden hamsters were used, from a commercial dealer or reared at our institute. They were fed standard pellets with milk and fresh greens. Newborn hamsters were implanted subcutaneously on the back with finely minced sarcoma tissue suspended 1:5 in Hanks' solution containing antibiotics, or with supernatant fluid. The injection needle was introduced through the right hind leg. The adult hamsters received sarcoma suspension or supernatant intramuscularly in the right thigh.

The injected hamsters were examined every other day for the first few weeks, then once a week or fortnight. The growth of the tumors was assessed by measuring them in three diameters with calipers. As a rule the hamsters were killed when it was suspected that they would not survive the night.

Results of Implanting Minced Chicken Sarcoma

Finely minced chicken sarcoma was injected into 26 hamsters. Two litters of newborn and one litter of 2-week-old animals were given 0.1 ml of sarcoma suspension subcutaneously in the back, while full grown hamsters received 0.5 ml of sarcoma suspension intramuscularly in the right thigh. In the beginning the animals seemed unaffected, and their growth was at the normal rate. However, tumors developed at the site of injection in almost all of them (Table I). The interval between injection and the appearance of tumor was 2 to 3 weeks in the hamsters injected at 2 or 4 days of age, somewhat longer in those injected at 14 days, and as long as 2 to 4 months in the full grown hamsters.

Gross Findings.—In the *young* hamsters the tumor appeared as a circumscribed, fairly soft, and later firmer, nodule on the back or right hip, usually adherent to the underlying tissue and covered by normal skin. It grew rapidly. During the following week new tumors often appeared in the injected area of the back, on the left side of the chest and/or in the right thigh (Fig. 1). The surface of the tumor was initially smooth but gradually became nodular. Some of the nodules felt soft, and almost fluctuating. Sometimes the skin became adherent to the surface of the tumor, and over the soft nodules it often turned dark red and became tense and finally ulcerated (Fig. 2). The growth assumed considerable dimensions within a few weeks and severely crippled the hosts. In some cases the weight of the tumors was as much as one-fourth of the total weight of the host. Most of the hamsters inoculated soon after birth survived only 1 to 2 months, though 2 survived 3 months.

In the *adult* hamsters the tumors were localized to the site of inoculation in the right leg or right hip. The tumors grew slower than in the young animals and they felt firmer. They often grew to twice the size of a walnut and the hosts succumbed within 1 to 2 months of their appearance (Fig. 3).

At postmortem examination the tissue around the tumor was edematous and showed small, scattered hemorrhages. Sparsely, disseminated, grain-sized, yellow-white, firm granules were seen subcutaneously on the back. Some of the tumors were fairly firm, others soft. The cut

surface was varicolored: the central areas were yellow-white or yellow-red, necrotic, and dry, while a peripheral area was grey-white, moist, and evidently made up of living neoplastic tissue. The necrotic area sometimes showed hemorrhages with cystic softening, corresponding to the soft protuberant nodules felt *in vivo*. Small, focal calcigerous deposits were found in the necrotic parts of the tumor, particularly in the tumors of the thigh.

Tumors in the back often grew into the abdominal cavity, tumors in the hip into the pelvis (Fig. 4). In these cases, the abdominal cavity sometimes contained a few milliliters of hemorrhagic fluid, and the peritoneum sometimes showed disseminated small tumor nodules which were best seen in the omentum, the mesenterium, or on the underneath side of the diaphragm. Sometimes the spleen and the pancreas were surrounded by soft, grey-red tumor masses, and in one hamster the kidney and the suprarenal on one side were embedded in tumor tissue. The retroperitoneal lymph nodes were often enlarged, grey-white, and involved by tumor growth (Fig. 4). No tumor nodules were seen in the liver, spleen, or kidneys. On the other hand, the

TABLE I

Hamsters Implanted with Minced Rous sarcoma

No. of hamsters	Age at inoculation	The first sarcoma after	No. of hamsters with sarcomas
6	2 days	14 days	6/6
7	4 "	14 "	7/7
8	14 "	20 "	5/8
5	Full-grown (100 gm)	64 "	5/5

lungs often showed small, round grey-red or grey-white tumor nodules, which were either deep-seated or protruded above the surface of the lungs. The mediastinal lymph nodes were often infiltrated with tumor.

No hemorrhages were found apart from hemorrhages in the tumors and in their immediate surroundings. No cysts were seen in the groins, axillae, or other lymph node stations. Multiple, circumscribed yellowish-white necroses were found in the liver of a few of the animals. None of the other organs showed anything noteworthy.

Microscopic Findings.—The tumors were usually pleomorphic and were dominated by large, rounded, or irregular cells with one or two, rarely several, nuclei and rather rich cytoplasm (Fig. 5). They were usually loosely arranged and surrounded by numerous, small cells; sometimes the large cells were rather closely packed and separated only by sparse connective tissue fibrils and single, small round cells (Fig. 6). The nuclei in the large cells had one or two distinct nucleoli and unevenly distributed chromatin. The nuclear membrane was slightly wrinkled. Usually the nucleus or nuclei were located in the periphery of the cell. In the central part of the cell the cytoplasm sometimes appeared to be homogenous and stained pale-red in eosin, while the peripheral part of the cytoplasm appeared finely granular and showed a faint bluish-violet hue in hematoxylin-eosin-stained specimens. The cytoplasm often showed small vacuoles in the periphery. The homogenous central part of the cytoplasm was sometimes fairly distinctly separated from the periphery (Fig. 7). Some cells had a monstrous appearance: they were elongated or racket-shaped and had numerous nuclei (Fig. 8). Their cytoplasm was sometimes striated longitudinally. Cross-striations could, however, not be demonstrated.

The small cells, which were intermingled with the above-mentioned large cells, had round or slightly irregular nuclei, rich in chromatin, and only scanty cytoplasm. They seemed to be

almost regular accompaniments of the large cells. Transitional forms between these and the small cells were sometimes seen. Some of the small cells, however, seemed to be stroma cells or lymphocytes.

Some tumors showed a different picture, that of a spindle cell sarcoma, built up of crowded, elongated cells (Fig. 9) arranged in bundles and with a varying amount of thin collagen fibrils. The cells were of 2 types (Fig. 10). One type had a narrow elongated, fairly chromatin-rich nucleus and sparse cytoplasm; the other, an oval nucleus with finely dispersed chromatin and a distinct nucleolus. These cells were richer in cytoplasm. The cells were irregularly intermingled, and transitional forms between the two types of cells were common. The features of a spindle cell sarcoma were most frequently seen in the thigh tumors, but were sometimes also observed in tumors on the back. Sometimes the appearance of one and the same tumor was pleomorphocellular in one area, but resembled that of spindle cell sarcoma in another. In some tumors giant cells and spindle cells were intermingled (Fig. 11).

The histological picture of the nodules in the lungs, in the lymph nodes, and on the peritoneum was largely the same as that described above. The earliest lung metastases appeared as a small number of large tumor cells, rich in cytoplasm and apparently filling a group of alveoli (Fig. 12). In more advanced cases (Fig. 13) the metastases showed the same polymorphocellular structure as the primary tumor with intermingled small and large cells. The larger, secondary growths showed a tendency to undergo necrosis. The lymph nodes were sometimes totally or partly invaded by tumor cells (Fig. 14) and usually showed a somewhat pleomorphic picture.

TABLE II

Newborn Hamsters Injected with Supernatant Fluid from Homogenized and Centrifuged Rous Sarcoma Tissue

No. of hamsters	Age at inoculation	The first sarcoma after	No. of hamsters with sarcomas
8	24 hrs.	13 days	7/8
6	12 "	13 "	5/6
6	2 mos.	4 mos.	3/6

The small yellowish granules in the neighborhood of the tumors microscopically showed a central necrosis with a tendency to undergo calcification. The necrotic areas were surrounded by a thick layer of connective tissue and seemed to be encapsulated remnants of the injected material.

Results of Injecting Supernatant Fluid from a Suspension of Homogenized Chicken Sarcoma

Supernatant fluid from homogenized chicken sarcoma tissue suspended 1:5 in Hanks' solution with antibiotics and repeatedly centrifuged in the way already described, was injected subcutaneously into the back of 2 litters of new-born hamsters and intramuscularly in the right thigh of six 2-month-old hamsters (Table II). Two animals were lost by cannibalism during the 1st week after the injection. The remaining animals grew at about the normal rate.

It is clear from the table that tumors appeared in almost all of the newborn hamsters injected with supernatant fluid. The latent period of tumor induction was somewhat shorter than after implantation with cellular material. Tumors appeared after 4 to 7 months in 3 of the 6 hamsters injected at 2 months of age. The tumors were localized to the injected area

and sometimes appeared along the whole track of the injection needle (Fig. 15). They increased rapidly in size and at postmortem examination they were often as large as 5 by 3 by 2 cm. Some hamsters were killed for histological purposes, the others died 2 to 4 weeks after the appearance of the tumors. Some hamsters, in which the skin over the tumors had ulcerated, were lost by cannibalism.

The tumors had the same variegated gross appearance as that described above. In many cases they invaded the abdominal cavity from behind and the peritoneal surface often showed disseminated small tumor nodules. In some of the animals metastases were seen in the lungs and in the retroperitoneal or mediastinal lymph nodes. The microscopic structure of the growths was the same as that of the tumors which developed after implantation with cellular material.

All attempts to induce sarcomas in newborn hamsters with Seitz filtrates of extracts of the chicken sarcoma failed.

Serial Transfer of the Hamster Sarcoma in Hamsters

Seven 1-day-old hamsters were injected subcutaneously on the back with 0.1 ml of a finely minced 16-day-old hamster tumor suspended 1:5 in Hanks' solution with antibiotics. Three weeks later the back of one of the injected animals showed a pepper corn–sized nodule, which gradually grew and after 6 weeks measured 4 by 4 by 2 cm. The major part of the tumor was necrotic, dry, and yellowish, apart from a narrow brim of living, moist, grey, sarcoma tissue. No metastases were seen. No tumors appeared in any of the other injected hamsters.

Living portions of the tumor were finely minced and suspended 1:5 in Hanks' solution with antibiotics, and 0.5 ml was injected intramuscularly in six 1-month-old hamsters. Takes were obtained in all of the animals within 12 to 14 days. The tumor has since been carried in series in new hamsters by injection at 2- to 4-week intervals with 0.2 ml of finely minced sarcoma, suspended 1:5. Takes have almost regularly been obtained in newborn as well as in 1- to 2-month old hamsters. The tumor has by now been passed through 9 generations. Secondary nodules have occasionally been seen in the lungs or in the lymph nodes.

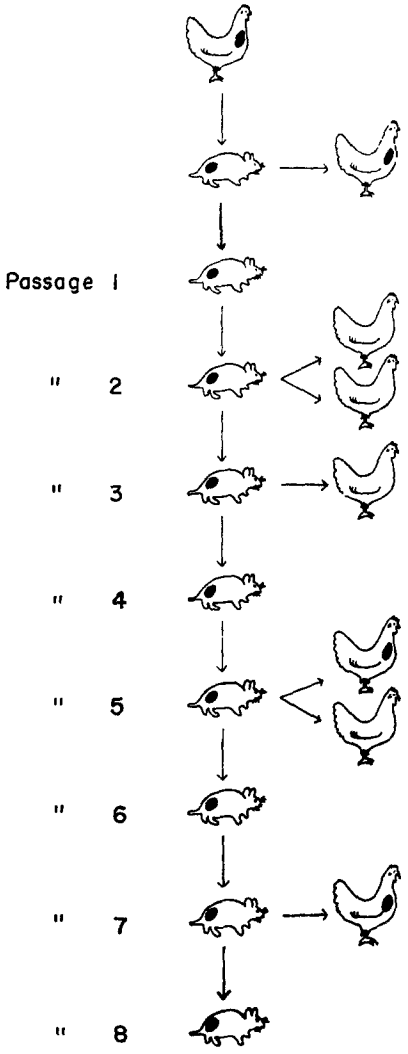
During the successive passages the histological picture of the series-transplanted tumor has become less variegated than that of the sarcoma from which it was derived. It is now composed of uniform cells with round or slightly oval nuclei and with a rather rich cytoplasm (Fig. 16). The nuclei have finely dispersed chromatin and one or two distinct nucleoli. Numerous mitoses are present. No inclusion bodies have been demonstrable. The central parts of the tumor usually show extensive necrosis. In the periphery the tumor cells can be seen to have invaded lymph or blood vessels (Fig. 17).

Numerous attempts to transfer the hamster sarcoma to hamsters by means of supernatant fluid from centrifuged hamster tumor-homogenates, suspended in Hanks' solution, have proved unsuccessful.

Back Transfer of the Hamster Sarcoma to the Chickens

The series of experiments performed to transfer the hamster sarcoma to chickens are summarized in Text-fig. 1.

In the first experiment the sarcoma appeared 14 days after injection of a 2-day-old hamster with Schmidt-Ruppin material. Sixteen days later, when the hamster was killed, the tumor measured 2 by 2 by 1.5 cm. Tissue removed from its living periphery was finely minced with



TEXT-FIG. 1. Induction of a sarcoma in hamsters with material from the Rous sarcoma, serial transfer of the hamster sarcoma in hamsters, and the result of transmission of the sarcoma from hamsters to chickens.

scissors, suspended 1:5 in Hanks' solution, and 0.2 ml of the suspension was injected into the pectoral muscles of a 3-week-old chicken. The bird died 5 weeks later and was then found to have a large greyish-white, partly slimy tumor in the region of the implantation. The gross and histological appearance of the tumor resembled that of a Rous sarcoma.

It is clear from Text-fig. 1 that a chicken-induced hamster sarcoma was transferred not only to another chicken but also to another hamster and was then car-

ried in series from hamster to hamster. Material from the 2nd and 3rd passages prepared as above was transferred to chickens but no takes were obtained in any of them during an observation period of 3 months. However, when the experiment was repeated with material from the 5th and the 7th passage, takes were obtained in two of the three injected chickens. The tumors appeared about 14 days after the injection and increased, first slowly, then rapidly.

The chickens died 3 to 4 weeks after the appearance of the tumor. The growth was by then about the size of a chicken's egg and was partly fibrous, partly soft and slimy. In one chicken metastases were seen in the heart, and in the lungs as also a single small nodule in the liver. Histologically the tumor was made up of elongated cells arranged in bundles, often separated by slimy areas, poor in cells (Fig. 18). In some areas two types of neoplastic cells could be distinguished, one with an elongated nucleus, rather rich in chromatin, and one with a round or polygonal, vesicular nucleus. Morphologically the tumor had the appearance of a Rous sarcoma.

The interval between the injection of the Schmidt-Ruppin material into the first hamster in the series and the transfer of material from the 7th hamster passage to the chicken was 9 months. The duration of latency of the chicken tumor induced by material from the first hamster-tumor was about the same as of the tumor produced by material from the 7th hamster passage.

DISCUSSION

It is known that heterologous tumors may grow in hamsters under certain conditions. Hence the possibility must be considered that the sarcomas induced in our hamsters were composed of chicken cells. This would appear to be excluded by the following observations:—

Sarcomas developed not only in newborn hamsters but also in adult hamsters whose capacity to produce antibodies had not been impaired by any form of pretreatment, such as Roentgen-ray irradiation or cortisone. The interval between injection and the appearance of the tumor in the full-grown hamsters was as long as 2 to 4 months; *i.e.*, a latency period much longer than that of heterologous transplants. The hamster sarcomas were not temporary like heterotransplanted tumors but grew progressively throughout a fairly long time until the animals succumbed. Some of the histological features of the hamster sarcomas differed considerably from those in chickens. Finally, sarcomas developed after the injection of supernatant fluid, obtained by repeated centrifugation of suspension of homogenized chicken sarcoma, and presumably cell-free.

In rat sarcomas, induced in our laboratory in the same way as the hamster sarcomas no serological evidence of any antigen common to the rat sarcoma and the chicken sarcoma could be demonstrated. The serological analysis was kindly done by Dr. I. B. Laurell, Bacteriological Institute, Lund, using a micromodification of the double diffusion-in-gel-method described by Wadsworth. No precipitation lines were observed in the gel between an extract from a Rous virus–induced rat sarcoma and anti-chicken sarcoma serum, prepared

by immunizing rabbits with a suspension of the chicken sarcoma. On the contrary a number of precipitation lines were seen in the gel between the anti-chicken sarcoma serum and an extract from the chicken sarcoma or from chicken liver. The results indicated that the cells from which the rat sarcomas were built up were not of chicken cell nature. Chromosome analysis of sarcomas in rats, mice, and guinea pigs induced by Schmidt-Ruppin material showed chromosomes that had the general appearance of rat, mouse, and guinea pig chromosomes, respectively. No cells with chicken chromosomes were found (A. Levan, data to be published).

There is no reason to assume that the hamster sarcomas should differ in these respects from the other sarcomas mentioned. These observations together with the well known fact that heterotransplanted tumors at the best show only a short temporary growth, clearly indicate that the hamster sarcomas are *not* built up by chicken cells.

We have tried many times to induce sarcomas in newborn rats, mice, hamsters, and guinea pigs with Seitz filtrates from the chicken sarcoma but have not met with any success, perhaps owing to too great a reduction of the amount of virus during the filtration. Svoboda (22) has already reported negative results of experiments with such virus extracts and he has discussed the possibility that a limited survival of transferred sarcoma cells permits a gradual adaption of the Rous virus to the rat or that transferred tumor cells induce a certain degree of tolerance to the virus. This does not seem likely, however, since we have not succeeded in eliciting sarcomas in mammals with Mill Hill strain of Rous virus.

It is noteworthy that in several respects the reaction of the hamsters was not the same as that of rats (3). Newborn rats inoculated with Schmidt-Ruppin material very often develop lymphogenous cysts situated in the groins, axillae, and neck and usually filled with a blood-stained fluid. No cysts were seen in the hamsters, nor did they show any hemorrhages apart from some hemorrhagic necroses in the sarcomas together with tiny hemorrhages in the immediate neighborhood of the tumors.

Adult hamsters have also proved susceptible, whereas full-grown rats have been completely refractory. In rats, injected soon after birth, the sarcoma did not appear until after an interval of at least 1 month, while the corresponding period in the hamsters was usually only 2 weeks. In hamsters the local growth of the tumor was just as invasive as in the rats, but metastases in the lungs and lymph nodes were much more common in the rats.

In some respects the histological picture of the sarcomas in hamsters resembled that of the tumor in the rats, but in others it differed considerably. The rat sarcomas usually have the character of a more or less undifferentiated spindle cell sarcoma. Some of the sarcomas in the hamsters showed the same picture and, as in the rat sarcomas, 2 types of cells can be distinguished: an

elongated type with a narrow nucleus, rich in chromatin and a more rounded type with a vesicular nucleus and finely dispersed chromatin. Usually, however, the sarcomas in the hamster are polymorphocellular and readily distinguished from those in rats. The giant cells in the sarcomas of hamsters sometimes became elongated and the cytoplasm striated, and then the tumor tended to resemble a rhabdomyosarcoma. Such pictures have never been seen in the rat sarcomas.

It is not possible to say with certainty whether the secondary tumor nodules seen in the lungs and lymph nodes of some of the hamsters were metastases due to disseminated tumor cells or whether they were induced *in loco* by some agent borne there by the blood or lymph stream. The distribution of the nodules showed the same pattern as that after the dissemination of tumor cells, and they were always much smaller than the tumor at the site of the injection.

The first attempt to transfer the hamster sarcoma to new hamsters proved successful in only one of the 7 animals injected. On further serial passage however, takes were obtained in almost all injected animals. This could be ascribed to adaptation of the tumor, an assumption supported by the observation that the serially transplanted growth assumed a more uniform histological picture than was presented by the polymorphocellular sarcoma from which it originated, and that the period of latency gradually became shorter. The rat sarcoma induced by Schmidt-Ruppin material had to be passed through several newborn rats before it could be successfully transplanted to adult rats, whereas the sarcoma of mice induced in the same way could be transplanted to full-grown mice from the very beginning (3, 4).

All attempts to transfer the sarcoma from hamster to hamster by injection of supernatant from centrifuged tumor homogenates have so far failed, as already stated. This also held true of the tumors induced in mice and rats by the Schmidt-Ruppin chicken sarcoma. It can perhaps be ascribed to an insufficient amount of oncogenic agent in the sarcomas, but it is also possible that the agent is "masked" or that its action is in some way inhibited. The fact that it was possible to transfer the tumor back to chickens can be explained on the assumption that since the birds are the natural host of the tumor, they are more susceptible to the oncogenic agent than mammals. It is possible that an increased concentration of the oncogenic agent, like that in SE polyoma, virus-induced tumors, may be obtainable by propagation of the tumor in tissue culture.

It proved possible to transfer the tumor back to chickens with material not only from the first Rous sarcoma-induced tumor in hamsters but also from the 5th and 7th passage tumor. Chickens inoculated with material from the 2nd and 3rd passages did not develop sarcoma. It seems probable that in these instances the material used for transplantation had been taken from an unsuitable part of the tumor. The sarcoma in the chickens, which developed at the

site of implantation, had the same character as the sarcoma with which the first hamster was injected, and in gross and microscopic appearance as well as in its general behavior in chickens it was indistinguishable from a Rous sarcoma.

Recent reports show that the Rous virus has lost the pathogenic specificity that it had when first isolated. Thus, Zilber and Kryukova (25) have reported the appearance of hemorrhagic cysts, and Svet-Moldawsky (18–20) the development of sarcomas in rats injected intra-embryonally with Rous sarcoma virus. Zilber has also reported (24) the appearance of 8 sarcomas among 151 rats injected with Rous sarcoma virus. In newborn rabbits the same virus elicited multiple fibrous nodules. Schmidt-Ruppin (15) transmitted Rous sarcoma to rats and mice and using the same material Ising-Iversen (11) induced sarcomas in rats and found that the sarcomas could be transferred back to chickens. Svoboda *et al.* (21–23) reported that newborn rats inoculated with Rous virus developed hemorrhagic cysts and that 2 out of 87 animals also developed sarcomas which could be carried in series in rats and which, even after 21 passages through these animals, could still be transferred back to chickens. Using the same Rous sarcoma material as in our hamster experiments, we have induced progressively growing sarcomas in rats, mice and guinea pigs and sarcoma-like lesions in rabbits (2, 3, 7). The tumors in rats and mice have been successfully transferred back to chickens.

All earlier reports had indicated that the Rous sarcoma virus cannot attack mammalian cells, a view which we were inclined to share on the basis of extensive experiments on newborn rats and mice inoculated with a Rous virus obtained from the Institute of Medical Research in London (Mill Hill strain) (2). It may be asked whether the enlarged scope of the virus is due to a contamination of the chicken sarcoma, in our experiments as well as in those of the Russian and Czech investigators, with some other virus, such as SE polyoma virus. No hemagglutinins indicating the presence of SE polyoma virus has been found however, in our chicken sarcoma material (3) nor in the induced rat, mice and hamster sarcomas. Though the possibility of contamination with some unknown virus cannot be wholly excluded, it seems most likely that the new findings are due to a variant in the strains of Rous sarcoma virus used in the different experiments.

Rous virus can be transmitted not only to chickens but also to a number of phylogenetically distant species of birds,—ducks (8), turkeys, guinea fowls (9), and pigeons. The existence of Rous virus strains capable of inducing sarcoma also in mammals is therefore perhaps not so very astonishing. It is not known with certainty which strains of Rous virus possess this property. The Mill Hill strain appears to be completely devoid of it (3), whereas the Schmidt-Ruppin strain has a very broad pathogenic spectrum. We are not in a position to say anything about the spectrum of the strains used by Zilber (24), Svet-Moldawsky (18–20) and Svoboda (22). Zilber reported that the strains of Rous virus used by him varied in their capacity to induce hemorrhagic disease in rats and fibromatosis in rabbits. Neutralization tests are needed to elucidate

the relation between the different strains of Rous sarcoma used in the various laboratories.

SUMMARY

Newborn hamsters were injected subcutaneously with a suspension of finely minced Rous chicken sarcoma (Schmidt-Ruppin strain). After an interval of about 2 weeks, progressively growing sarcomas developed at the site of injection in almost all animals. Also in adult hamsters inoculated intramuscularly with the same material sarcomas developed at the site of injection within 2 to 4 months. Secondary growths appeared on the peritoneal surface, in the retroperitoneal and mediastinal lymph nodes and in the lungs. The sarcomas usually had a pleomorphic appearance and showed a certain resemblance to rhabdomyosarcoma, but sometimes they had the character of spindle cell sarcomas of varying degree of maturity. Sarcomas were not only obtained in hamsters injected with cellular material from the Rous chicken sarcoma but were also seen in hamsters which were injected at birth or when 2 months' old with supernatant fluid obtained by repeated centrifugation of suspensions of homogenized chicken sarcoma, and presumed to be cell-free.

The hamster sarcoma was transplanted to a newborn hamster and could then without difficulties be passed in series in hamsters. All attempts to transfer the sarcoma from hamster to hamster by means of cell-free material from the hamster sarcoma failed. On the other hand, material from the hamster sarcomas inoculated into chickens induced rapidly growing Rous sarcomas at the site of inoculation. This proved possible not only with material from the first but also from later passages of the tumor in hamsters.

It is concluded that the strain of Rous virus used has the capacity to induce sarcomas not only in chickens but also in hamsters.

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EXPLANATION OF PLATES

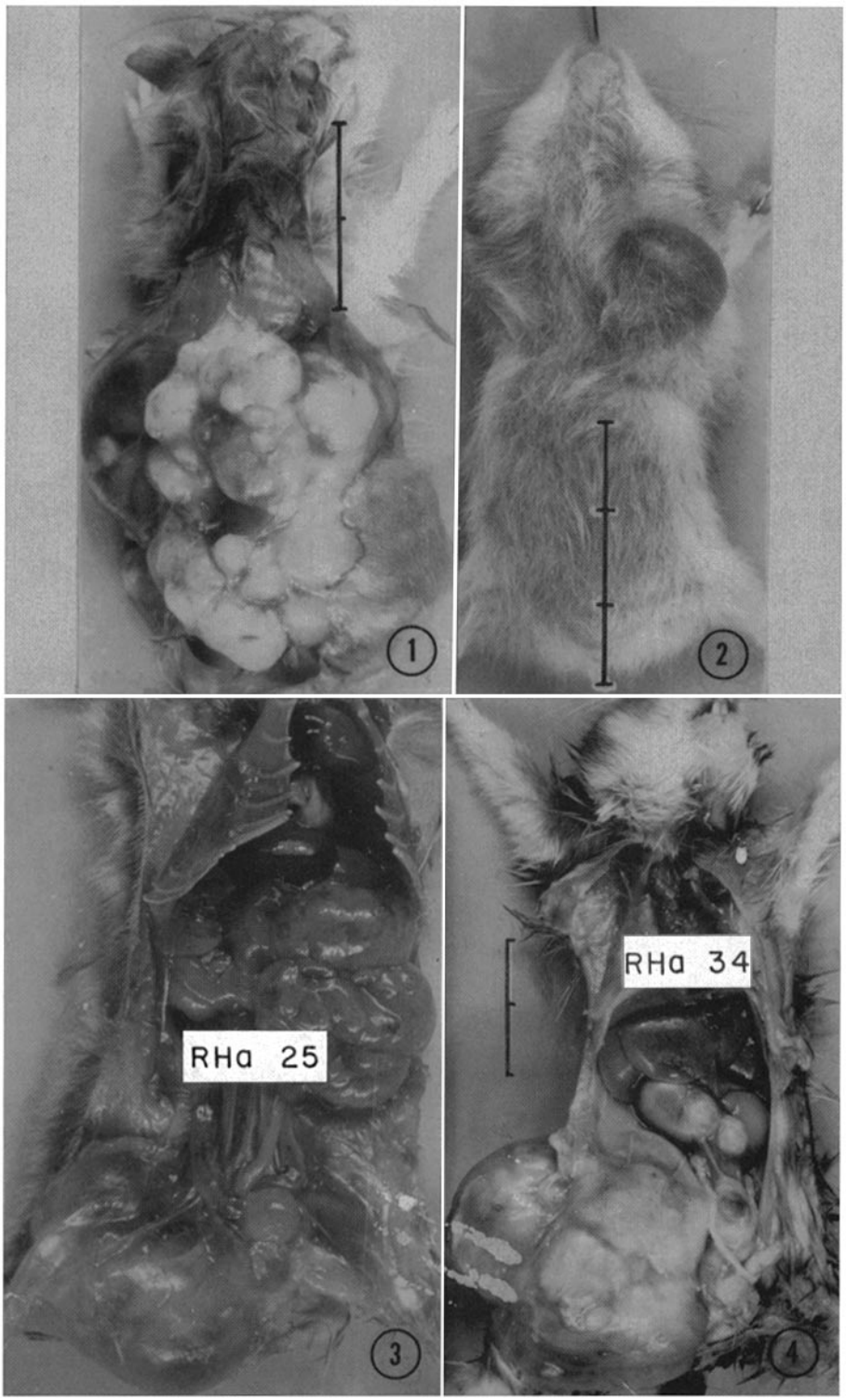
PLATE 65

FIG. 1. Nodular tumors in the right thigh and on the back of a 5 week old hamster injected at 2 days of age with a suspension of minced Rous sarcoma. × 1.

FIG. 2. One-month old hamster implanted at 2 days of age with Rous sarcoma tissue. A protuberant, fluctuating tumor nodule covered with red glistening skin is seen on the left side of the thorax. × 1.

FIG. 3. Sarcoma in the right thigh of a hamster injected when adult with a suspension of Rous sarcoma. × 1.

FIG. 4. Large growth situated in the right thigh and invading the pelvis, with metastases in the retroperitoneal lymph nodes, in a 3½-month old hamster injected at 14 days of age with a suspension of Rous sarcoma tissue. × 1.



(Ahlström and Forsby: Sarcomas in hamsters)

PLATE 66

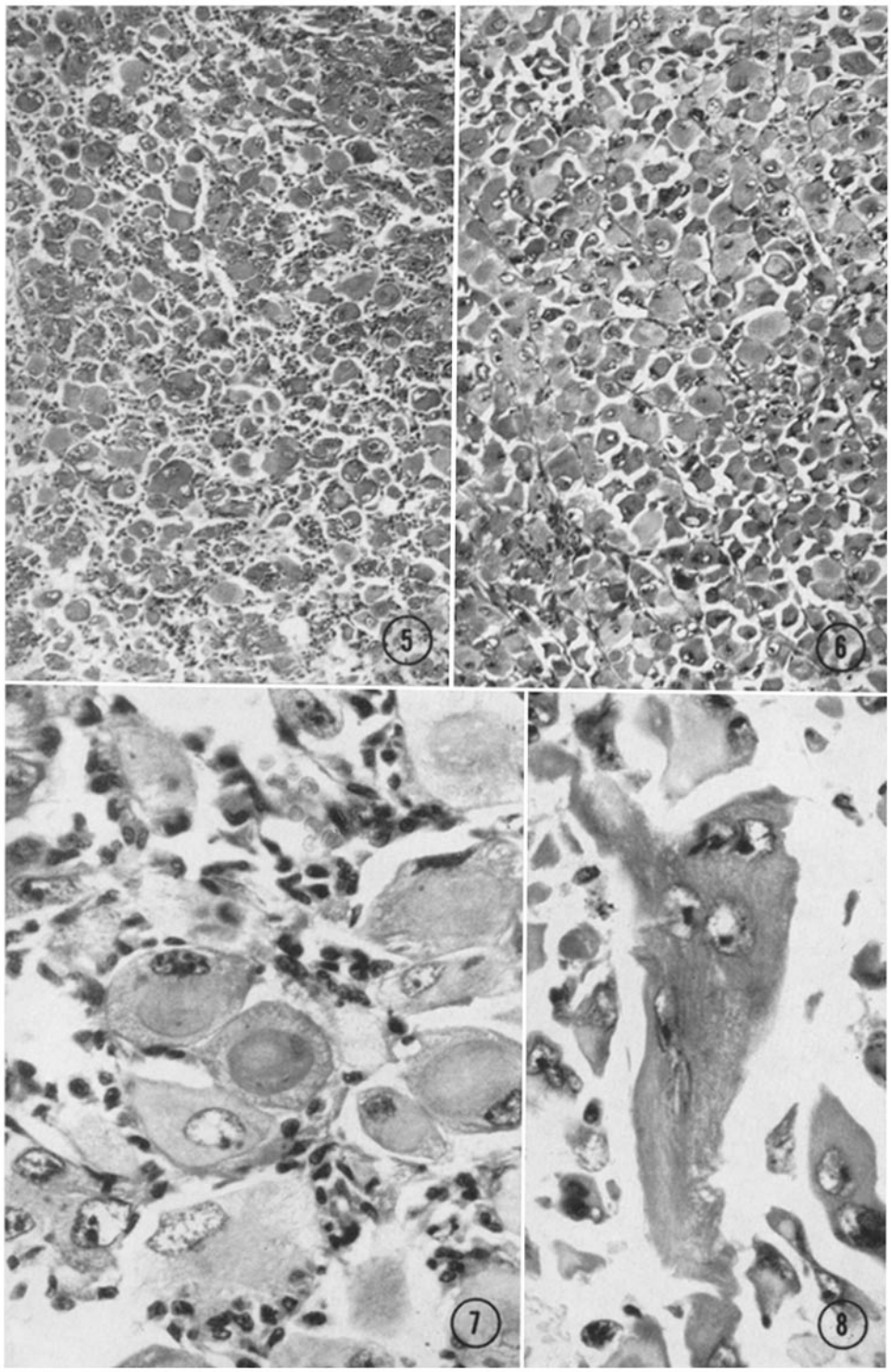
The sections were all stained with hematoxylin and eosin.

FIG. 5. Section of a sarcoma at the site of injection on the back of a hamster, showing the picture of a polypleomorphic sarcoma with large rounded or irregular cells surrounded by sparse connective tissue fibrils and numerous small cells. × 140.

FIG. 6. In some areas the picture is dominated by rather closely packed large cells. × 140.

FIG. 7. Large cells whose cytoplasm shows a central homogenization fairly well demarcated in the periphery. × 500.

FIG. 8. Elongated large cell with several nuclei and a striated cytoplasm. × 500.



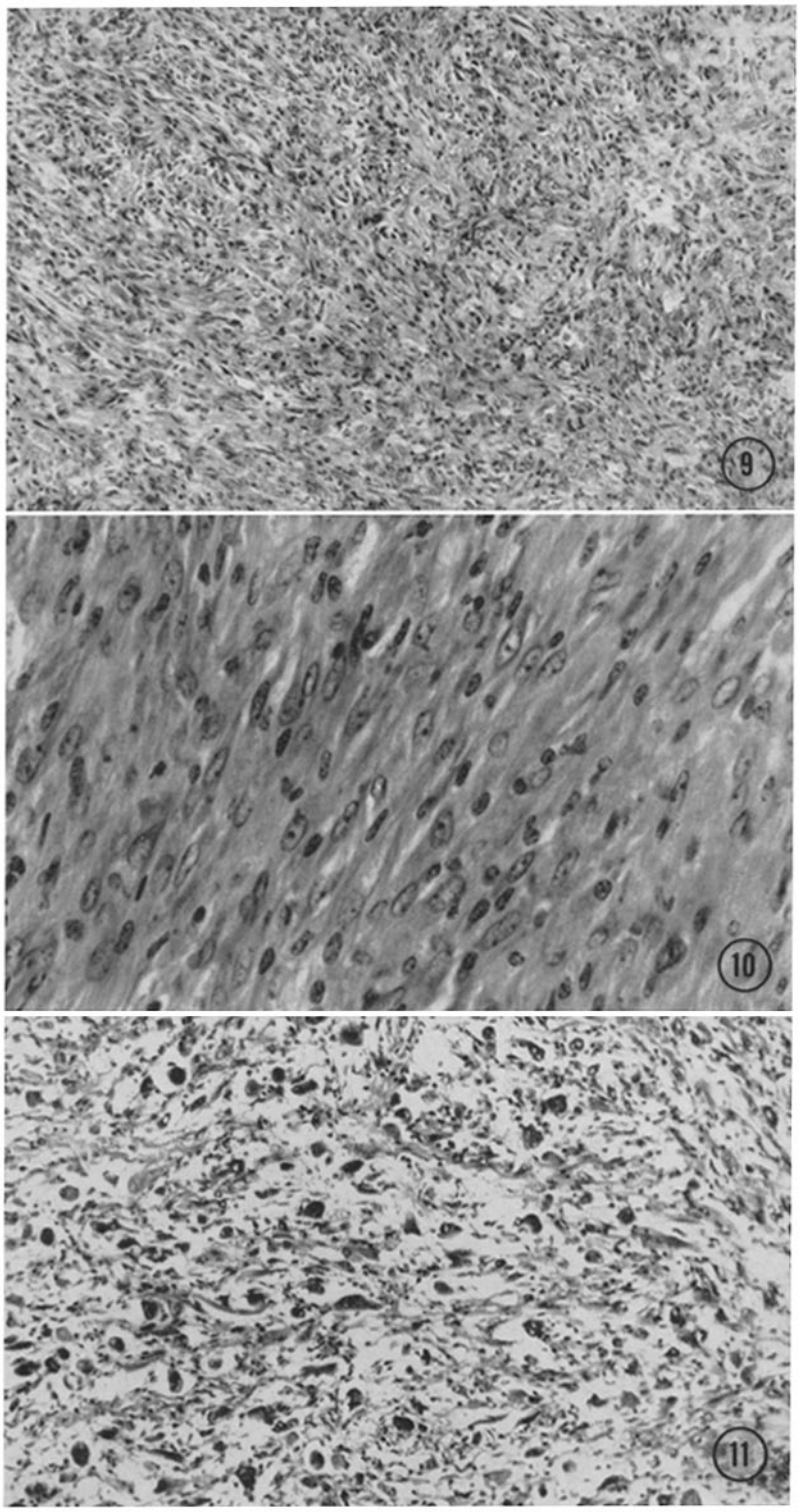
(Ahlström and Forsby: Sarcomas in hamsters)

PLATE 67

FIG. 9. Illustration of a hamster sarcoma with the character of a spindle cell sarcoma built up of elongated cells with a varying amount of connective tissue fibrils. × 140.

FIG. 10. Two types of cell can be distinguished in the spindle cell sarcoma. × 300.

FIG. 11. Tumors with giant cells and spindle cells intermingled. × 140



(Ahlström and Forsby: Sarcomas in hamsters)

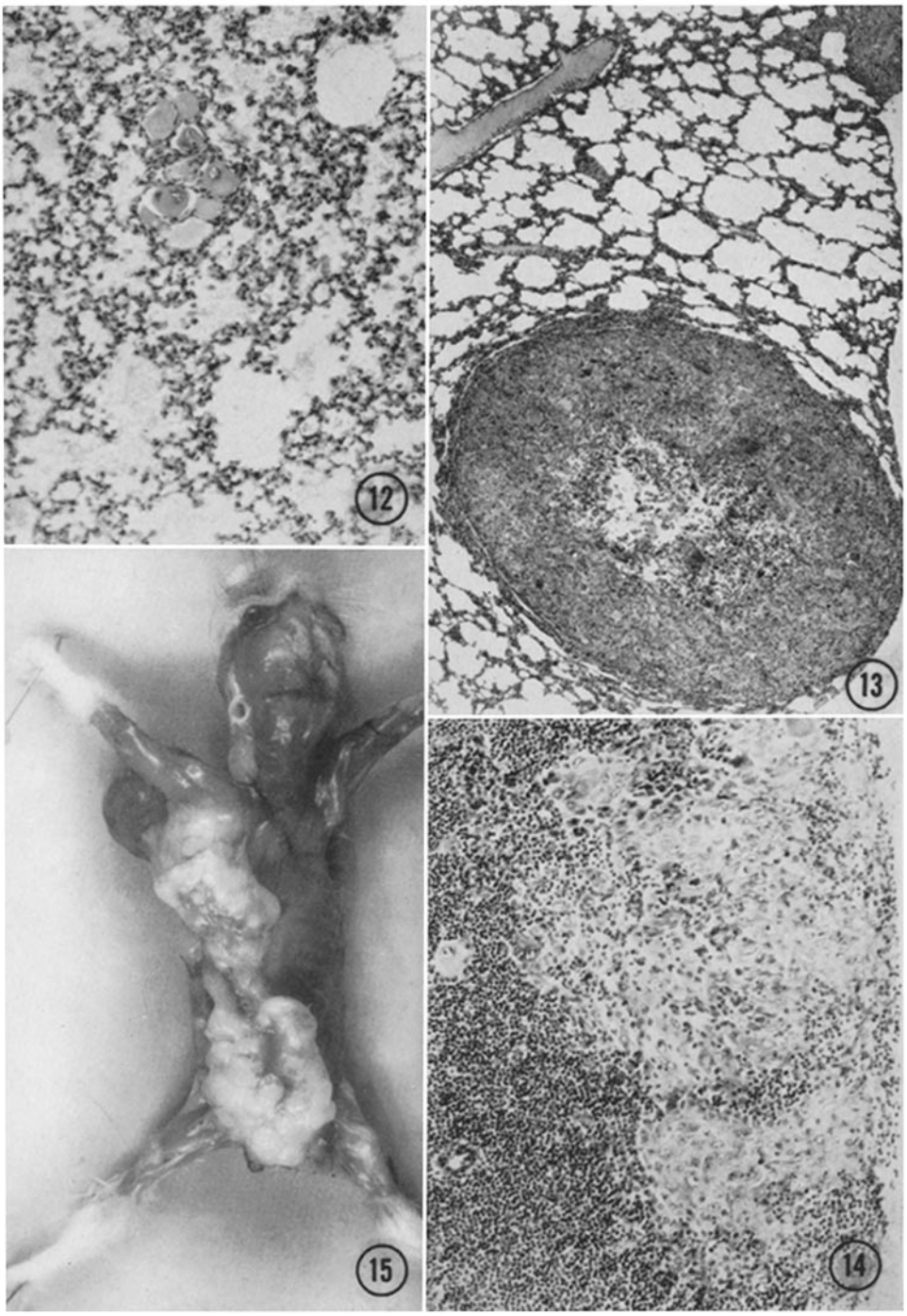
PLATE 68

FIG. 12. A group of large tumor cells in the lung. × 500

FIG. 13. Sarcoma nodules in the lung. × 40

FIG. 14. Sarcomatous growth in the periphery of a lymph node. × 140

FIG. 15. Chain of sarcoma nodules along the needle track in a month-old hamster injected subcutaneously at the age of one day with cell-free supernatant from homogenized, centrifuged Rous sarcoma material.



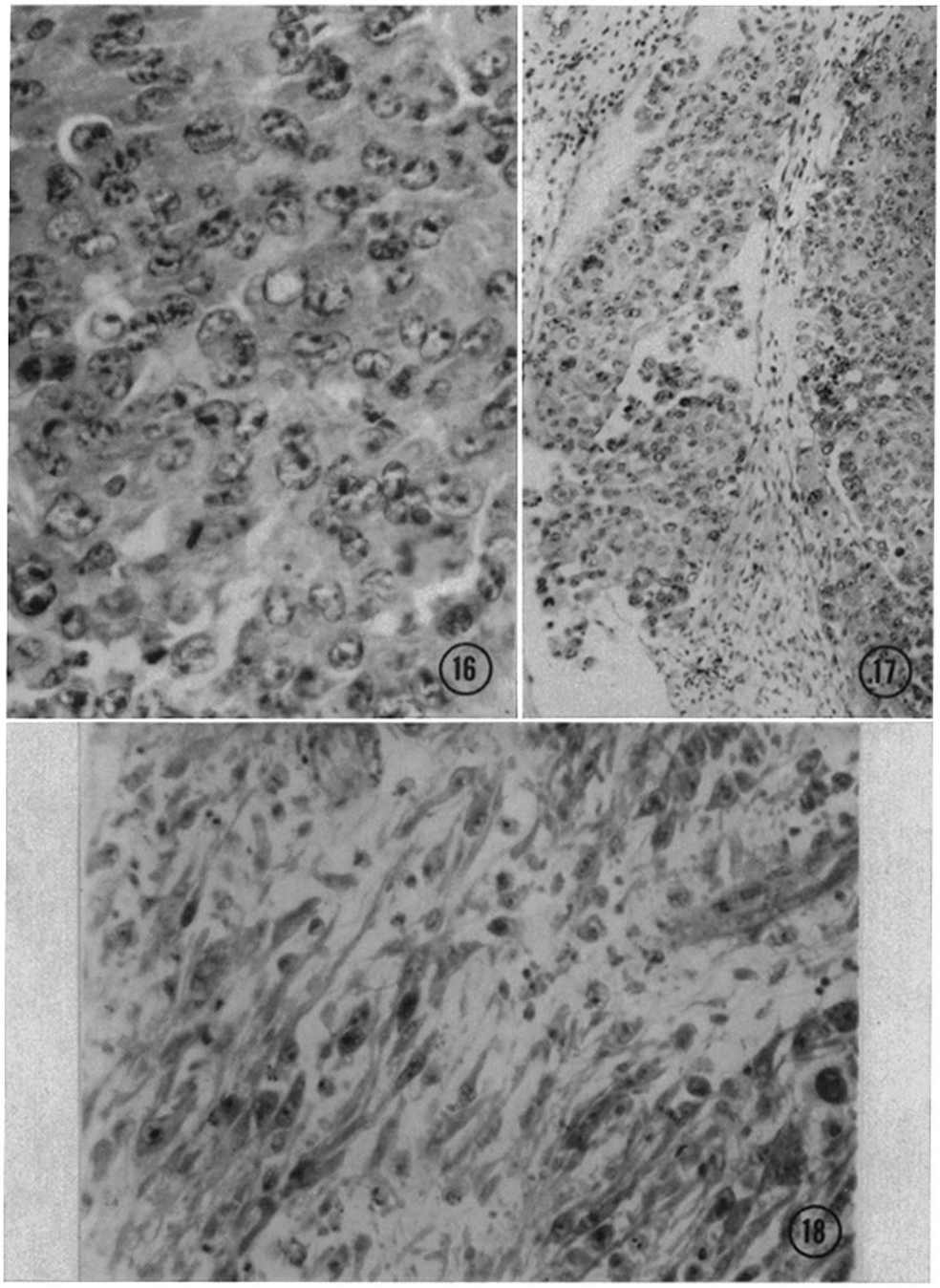
(Ahlström and Forsby: Sarcomas in hamsters)

PLATE 69

FIG. 16. Third passage of the hamster sarcoma. The tumor has a more uniform character than the sarcoma from which it was derived. × 500.

FIG. 17. Third passage of the hamster sarcoma. In the periphery of the tumor the cells are invading blood vessels. × 140.

FIG. 18. Sarcoma of a chicken after the injection of minced material from the 5th hamster passage tumor. The sarcoma is built up of loosely arranged, elongated cells and has the usual appearance of a Rous sarcoma. × 140.



(Ahlström and Forsby: Sarcomas in hamsters)