

6. T. Caspersson, *Skand. Arch. Physiol.*, Suppl. 8, 73, (1936); T. Caspersson and J. Schultz, *Nature* 142, 294 (1938); T. Caspersson and J. Schultz, in *Genetics in the Twentieth Century*, L. C. Dunn, Ed. (Macmillan, New York, 1950), p. 155; G. T. Rudkin, J. F. Aronson, D. A. Hungerford, J. Schultz, *Exptl. Cell Res.* 9, 193 (1955).
7. Preliminary results of this investigation were presented at the 1962 meeting of the Genetics Society of America in Corvallis, Oregon, and published in abstract form (G. T. Rudkin, D. A. Hungerford, P. C. Nowell, *Genetics* 47, 981, 1962).
8. Chromosomes in the 6-X-12 group could not be assigned to homologous pairs in these preparations on the basis of morphology, nor were the differences between them sufficiently great with respect to total absorbance, either in whole chromosomes or in individual arms, to assist materially in the identification of homologs. Translocation between a member of the 6-X-12 group and number 21 would not be detectable in this material.
9. L. T. Carlson, T. Caspersson, G. E. Foley, J. Kudynowski, G. Lomakka, E. Simonsson, L. Sörén, *Exptl. Cell Res.* 31, 589 (1963).
10. G. T. Rudkin, unpublished.
11. See, for example, D. A. Hungerford and M. DiBerardino, *J. Biophys. Biochem. Cytol.* 4, 291 (1958).
12. Contamination by cellular protein overlying metaphase plates or filling the spaces between chromosomes is a possible source of error in ultraviolet microspectrophotometric measurements of this material and was considered to be a major one in reference 9. Determination of the magnitude of the error (and correction for it) is experimentally possible, but laborious by the photographic method. If, however, we make the reasonable assumptions that (i) the mass per unit area of the contaminating protein is that of the chromosomal DNA and (ii) that the combined specific and non-specific absorptivity of the protein (for example, serum proteins) is 0.1 to 0.01 that of DNA at 257 m μ , then the possible error introduced by the protein would be only 1 to 10 percent. The twofold variation from cell to cell recorded in Table 1 would require the unlikely range of 20:1 or 200:1 in the ratio of mass per unit area of protein over chromosomes to that between chromosomes. Furthermore, if protein contamination were the major source of variability it would have to be related to the effect of pretreatment in uncoiling the chromosomes. We consider contamination by cellular proteins to be a minor source of variability in the measurements reported here, but reserve final judgment until definitive tests are made.
13. Calculated from spectrophotometric measurements of a highly polymerized *Drosophila melanogaster* DNA preparation in dilute solution, courtesy of Miss E. Travaglini of The Institute for Cancer Research, Philadelphia.
14. Integrated absorbance may be thought of as the product of the mean of the absorbance (of a chromosome) and the area (of the chromosome); its units are therefore those of area, which we express in square microns, multiplied by the dimensionless units of absorbance. The quotient of an observed A_T divided by the known absorptivity of an absorbing material, expressed in appropriate units, gives the mass of that absorbing material that would have the measured A_T . Thus, the A_T for nucleus 17-4 in Table 2 (28.54 μ^2), divided by the specific absorptivity at 257 m μ for one picogram per square micron of DNA (2.6), gives a value of 10.9 pg for DNA in that nucleus.
15. J. N. Davidson, I. Leslie, J. C. White, *Lancet* 1951-I, 1387 (1951).
16. See, for example, H. Swift, *Intern. Rev. Cytol.* 2, 1 (1953).
17. Aided by grants from NIH, from NSF, and by a career research development award (to P.C.N.) from the Public Health Service. The assistance of Carolyn Gibson and James A. Benner, Jr., in the tedious densitometric procedure is gratefully acknowledged.

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After a period of 2 to 8 weeks, a hard lump or several small coalescing nodules appeared at the site of injection in 11 of the animals. Most of the tumors grew rather slowly, gradually assuming, during the following months, the size of a hazelnut or walnut (Fig. 1). The underlying muscles were often invaded and the skin over the tumors sometimes became ulcerated. Often the tumors became softer during their growth. Two hamsters with tumors died 3 to 5 months after inoculation. Nine animals with tumors were killed at various intervals for histopathologic and chromosome study. Of the remaining four hamsters, one was eaten by the others and the remaining three did not show any tumor during an observation period of 6 months.

The tumors showed a moist, grayish-white, cut surface and sometimes a central softening or irregular grayish-red necrosis. Gross examination revealed pinhead-size metastases in the lungs of the two hamsters that died.

Microscopically the tumors showed the picture of a spindle-cell sarcoma (No. RCh H 4, Fig. 2a) or a polymorphous sarcoma. Numerous mitoses were observed. One tumor was similar to a human giant-cell tumor in bone; another to a hemangiosarcoma with numerous, closely packed, thin-walled vascular spaces, surrounded by polynuclear giant cells (No. RCh H 2, Fig. 3a). In a third hamster the tumor tended to simulate a malignant angioendothelioma.

One of the tumors was transplanted to the cheek pouches of two adult Chinese hamsters, one of which had been given 0.25 mg of hydrocortisone subcutaneously. Three weeks later a small tumor developed in the cortisone-treated animal but it soon regressed. The same tumor was also transplanted into the cheek pouches or into the legs of 14 Syrian hamsters (11 days old), some of which had been treated with hydrocortisone. Tumors developed in two of the cortisone-treated animals, and in two of the untreated animals. The tumors appeared about 11 days after the inoculation, grew very rapidly, but regressed spontaneously, disappearing after 2 more weeks. However, material from still vital tumors was successfully transferred by inoculation to new Syrian hamsters and carried in series through four passages. The transplanted tumors had the character of an anaplastic spindle-cell sarcoma with numerous mitoses. Several attempts were made to study the

Rous Sarcoma in Chinese Hamsters

Abstract. *A variant of the Rous sarcoma virus induced tumors in newborn Chinese hamsters within 2 to 8 weeks. The tumors grew progressively and sometimes metastasized. They were successfully transplanted in series in Syrian hamsters. The chromosomes of the tumors in the Chinese hamsters as well as of those transplanted into the Syrian hamsters were Chinese hamster chromosomes. Virus was demonstrated in the Chinese hamster tumors by injecting material from the tumors into chickens, where Rous sarcomas subsequently appeared.*

In the course of a systematic chromosome study of tumors induced in different rodents by the Rous chicken sarcoma virus it was considered important to obtain such tumors for chromosome analysis in the Chinese hamster (*Cricetulus griseus*). This species is characterized by a low chromosome number ($2n = 22$) and an unusually clear karyotype (1). Some strains of Rous sarcoma virus are known to produce rapidly growing and metastasizing sarcomas in the Syrian hamster (*Cricetulus aureus*) (2), but this species has double the number of chromosomes ($2n = 44$) and is considerably less favorable cytologically.

The breeding nucleus for a Chinese hamster colony, seven females and five

males, was kindly provided by George Yerganian. By applying the breeding procedures recommended by him (3), the propagation of the animals was successful. Our colony now totals more than 200 animals.

The Rous virus was of the same strain (strain Schmidt-Ruppin) as that used in previous experiments with Syrian hamsters, rats, mice, guinea pigs, and rabbits (4).

Fifteen Chinese hamster babies, 1 or 2 days old, belonging to three litters were injected subcutaneously in the back with material either from a pool of homogenized chicken sarcoma stored at -65° to -68°C , or from a suspension of freshly prepared and finely minced chicken sarcoma.



Fig. 1. Four-month-old Chinese hamster inoculated at 1 day of age with Rous sarcoma material. A protuberant nodular tumor has developed at the site of injection.

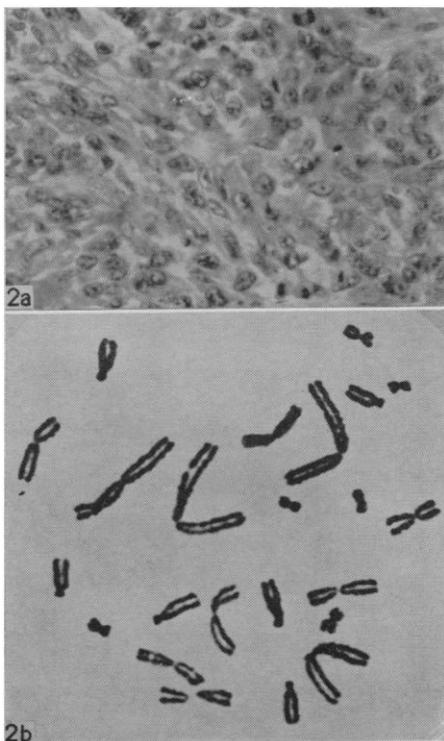


Fig. 2. *a*, Chinese hamster sarcoma with the appearance of an anaplastic spindle-cell sarcoma (No. RCh H 4, $\times 375$). *b*, Stemline karyotype of No. RCh H 4. Normal female karyotype with an extra chromosome among the medium-sized chromosomes with submedian centromere. In the cell pictured, one member of the longest pair shows a chromatid break. The centromeric region of one of the smallest chromosomes of the medium-sized group (at the 6-o'clock position) has become stretched by the squashing giving the appearance of fragmentation in this region ($\times 1150$).

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chromosomes of the original tumors in Chinese hamsters and after transplantation of the tumors to Syrian hamsters. In both cases, the characteristic Chinese hamster chromosomes were seen.

Good chromosome preparations were obtained from two primary tumors in Chinese hamsters—No. RCh H 2 and No. RCh H 4; the sections were obtained and fixed when the tumors were about 5 and 6 months old, respectively. In both tumors the predominant stemline differed from the normal karyotype of the species. In No. RCh H 4 the majority of cells were trisomic ($2n = 23$) for one of the medium-sized metacentric chromosomes (Fig. 2*b*). No structurally new chromosomes were seen in this tumor. In the other tumor, No. RCh H 2, a near-tetraploid karyotype prevailed with chromosome numbers varying from 38 to 47, the most common number being 42. New structural chromosome types had entered into the stemline, the most striking marker chromosome being a long metacentric with subterminal centromere (Fig. 3*b*, arrow). In two other primary tumors from which only mediocre slides were available, a few normal karyotypes with 22 chromosomes were seen. This was the case also with the tumor transferred to Syrian hamsters. Thus, the two primary tumors that could be studied in detail had both acquired heteroploid stemlines, one in the near-diploid, the other in the near-tetraploid chromosome number region. Only in the near-tetraploid were there signs of structural variation in the development of the stemlines.

Suspensions from a 2-month-old hamster tumor were inoculated into the pectoral muscles of two chickens. Three weeks later both showed rapidly growing tumors at the site of inoculation with the usual picture of a slimy hemorrhagic Rous sarcoma.

The reaction of the Chinese hamsters to the Rous sarcoma virus differs from that of the Syrian hamsters in the following respects. (i) The Chinese hamster was somewhat less susceptible than the Syrian hamster. While all the newborn Syrian hamsters developed tumors (2), they developed in only 75 percent of the newborn Chinese hamsters. (ii) Tumors appeared later and grew more slowly in the Chinese hamsters than in the Syrian hamsters. Metastases appeared rather late in the Chinese hamsters and were seen only in the lungs whereas the

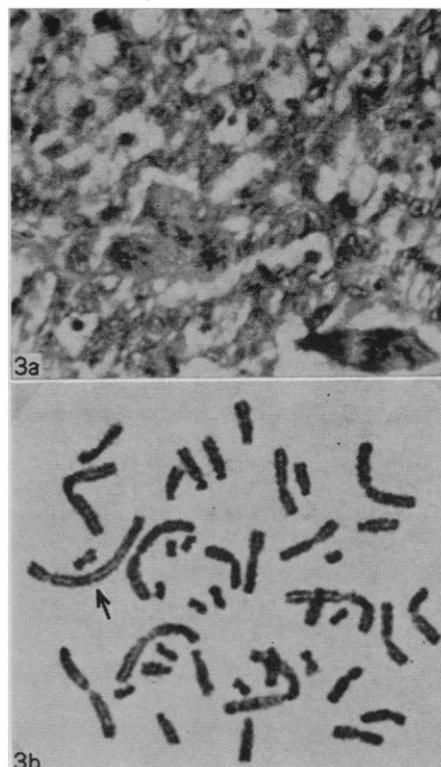


Fig. 3. *a*, Chinese hamster sarcoma with the appearance of a hemangiosarcoma (No. RCh H 2, $\times 375$). *b*, A 42-chromosome karyotype from the stemline of No. RCh H 2. This is a hypotetraploid male karyotype with a long marker chromosome with subterminal centromere (arrow) ($\times 1150$).

Syrian hamsters almost always showed metastases in the lymph nodes and lungs 6 to 8 weeks after inoculation. (iii) The sarcomas of the Chinese hamsters often differed histopathologically from the usually rhabdomyosarcoma-like growth in the Syrian hamsters. In both species the virus was recovered by injection of tumor material into chickens.

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