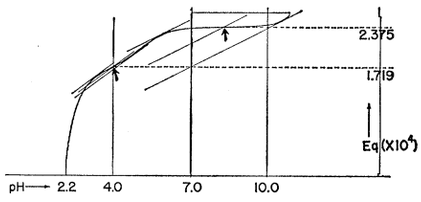

Fig. 1. Original titration chart obtained with 41.6 mg of heparinic acid prepared from sodium heparinate (159 units per milligram) and titrated with 0.1048*N* NaOH. Superimposed are *p*H values on the horizontal axis and equivalents of base represented by vertical distances above the starting line. Tangential lines constructed to determine sulfate and carboxyl end points (indicated by arrows) are also shown.

arin on the column was eliminated by subsequent passage of a tenfold excess, over heparin, of 4*N* HCl. The neutralized effluent gave negative orcinol and anthrone reactions.

Helbert and Marini (*8*) recently found a 2.7 percent increase in acid titratable groups of heparinic acid prepared from dialyzed sodium heparinate upon standing at 60°C for 1½ hours. To determine the extent of autohydrolysis resulting from the ion-exchange procedure we dialyzed two different heparin preparations for extended periods through cellophane (*13*) membranes. This initial dialysis removed about one-fifth of the original heparin. The 80 percent remaining in the retentate was passed through Dowex-50 W resin, and again dialyzed at 25°C. Titration of the second dialysate after one hour (when dilute sulfuric acid would have come to equilibrium) indicated loss of only about 2 percent of the total acid groups present, presumably in the form of macromolecular subunits or free sulfuric acid resulting either from autohydrolysis or from exchanged inorganic sulfate. Since the diffusion of 20 percent of the heparin during the initial dialysis indicated the probable presence of a mixture of heparins of different molecular weights, much as shown earlier by Barlow *et al.* (*10*), we decided to conduct our titrations on the original undialyzed material to avoid possible alterations in biological activity resulting from such a separation.

Each heparin, made up to about 0.5 percent in water, was passed through a cation exchange column as previously described. Equal portions containing about 60 mg of heparinic acid were immediately diluted to 20 ml with water and titrated with 0.1*N* NaOH. After passing the second equivalent point (*p*H range 11 to 13) the NaOH burette was turned off and the excess base immediately back-titrated with 0.1*N* HCl to *p*H 7. A typical titration curve is shown in Fig. 1.

Heparin is very hygroscopic, and after 3 to 10 minutes exposure to air after lyophilization, or vacuum storage over P_2O_5, or both, may increase up to 10 percent in weight. To avoid this problem the heparin solution was quantitatively transferred, immediately after titration, into a tared, lightweight lyophilization flask equipped with a high-vacuum teflon stopper. The solution was then lyophilized for 48 hours at a pressure of approximately 0.3 mm Hg, and the dried heparin weighed by difference.

The dry weight of each titrated heparin sample, obtained after titration and lyophilization, was used for the calculation of equivalent weight, corrections being made for the weight of additional NaCl formed during back-titration and for conversion of the heparinic acid to the sodium form. This weight was then divided by the total equivalents of base used for neutralization to give the equivalent weight (acid equivalent) of the heparinic acid. As shown in Table 1, the equivalent weights of the different heparin preparations averaged 170.0, including the material giving the lowest value, which was derived from beef lung. There was no significant correlation between bioactivity and equivalent weight. Figure 1 shows that the continuous titration procedure provides a sharp inflection point at which sulfate groups are titrated to R-SO_3Na, so that by midtangential construction, values for the total titratable $-SO_3H$ groups may be calculated. Individual values, given in Table 1, averaged 34.44 percent for sulfate ($-SO_3H$) and 2.59 for sulfate to carboxyl ratio. Again there was no notable relationship between the sulfate content, sulfate to carboxyl ratio, and the bioactivity. These ratios may be uncertain because of possible interference in the sulfate titrations by carboxyl groups. Improvement in the accuracy of the sulfate end point may be possible by such techniques as use of a first derivative circuit in the recorder or alteration of the dielectric constant of the solvent (*14*).

The large fraction of dialyzable heparin in our samples would also suggest that in the future, attempts should be made to correlate biological activity with parameters, such as equivalent weight or sulfate to carboxyl ratio, in more physically homogeneous material. However, the technique described herein provides perhaps the most accurate values for commercial heparin so far reported.

KLAUS E. KUETTNER*
ARTHUR LINDENBAUM
Division of Biological and Medical Research, Argonne National Laboratory, Argonne, Illinois

References and Notes

1. L. B. Jaques and H. J. Bell, *Methods Biochem. Analy.* **7**, 253 (1959).
2. U. Lindahl, *Federation Proc.* **22**, 413 (1963).
3. I. Danishefsky, H. B. Eiber, E. Langholtz, *J. Biol. Chem.* **237**, 1413 (1962).
4. O. Wilander, *Skand. Arch. Physiol.* **81** (Suppl. 15), 25 (1939).
5. M. L. Wolfrom, R. Montgomery, J. V. Karabinos, P. Rathger, *J. Am. Chem. Soc.* **72**, 5796 (1950).
6. R. A. Gibbons and M. L. Wolfrom, *Arch. Biochem. Biophys.* **98**, 374 (1962).
7. P. Hoffman and K. Meyer, *Federation Proc.* **21**, 1064 (1962).
8. J. R. Helbert and M. A. Marini, *Biochemistry* **2**, 1101 (1963).
9. H. J. Bell and L. B. Jaques, *Bull. Soc. Chim. Belges* **65**, 36 (1956).
10. G. H. Barlow, N. D. Sanderson, P. D. McNeill, *Arch. Biochem. Biophys.* **84**, 518 (1961).
11. W. R. Jeanloz and E. Forchielli, *J. Biol. Chem.* **186**, 495 (1950); I. Danishefsky, H. B. Eiber, J. J. Carr, *Arch. Biochem. Biophys.* **90**, 114 (1960); T. C. Laurent, *ibid.* **92**, 224 (1961).
12. These samples were obtained through the courtesy of H. H. R. Weber and C. de Fiebre of Wilson Laboratories, Chicago, Ill.
13. Obtained from the Visking Co., Chicago, Ill.
14. C. W. Pifer, E. G. Wollish, M. Schmall, *Anal. Chem.* **25**, 310 (1953).
15. Work supported by the U.S. Atomic Energy Commission. The assistance of Mrs. E. S. Moretti and Mr. William Eisler is gratefully acknowledged.
* Present address: Department of Orthopedic Surgery, Presbyterian-St. Lukes Hospital, Chicago, Ill.

15 April 1964

DNA Contents of Chromosome Ph[1] and Chromosome 21 in Human Chronic Granulocytic Leukemia

Abstract. *The Philadelphia[1] chromosome, characteristic of chronic granulocytic leukemia, contained 61±1 percent as much DNA as a number 21 (or 22) chromosome from which it is believed to be derived. The remaining 39 percent represents 0.5 percent of a diploid chromosome complement, approximately 2 × 10⁷ nucleotide pairs; the method used was not sensitive enough to detect whether it had been translocated to other chromosomes.*

Chronic granulocytic leukemia (CGL), the first of the neoplastic diseases shown to be associated with a constant abnormal karyotype, is unusual among human disorders associ-

ated with anomalies of autosomal chromosomes in that a portion of a chromosome appears to be lacking. In short-term cultures of leukemic cells from patients with this disease, mitotic figures have a chromosome of the 21–22 group (*1*) replaced by a smaller chromosome, presumably derived from a chromosome 21 by loss of much of the long arm (*2*). The time at which the change occurred is unknown, but it is inferred to have been in hematopoietic tissue because the atypical nuclei have not been observed in other tissues. The portion missing from this small chromosome, called the Philadelphia[1] (Ph^1) chromosome, may be lost, thus creating a deficient nucleus; or it may have become attached to another chromosome of the complement, in which case a near-normal amount of genetic material has been rearranged by translocation in the atypical cells. The distinction between deficiency and translocation in this case can only be made cytologically by detecting the lost portion of chromosome 21 elsewhere in the genome. This analysis is out of the range of current cytological methodology (*2*). The present work is an exploration of the possibility of answering the question by determination of the DNA content of the individual chromosomes, in the course of which we determined the relative DNA contents of all chromosomes in the karyotype.

Chromosome preparations were obtained from a 68-year-old white female (case 200 T) in the early stages of the disease. Her leukocyte count was 98,000 (25 percent blast cells and promyelocytes, 26 percent myelocytes), and she had been under treatment with Myleran for 8 days. Leukocytes from the peripheral blood were cultured for 2 days in normal human plasma without phytohemagglutinin [the presence of which is not necessary to stimulate mitotic activity of leukemic leukocytes (*3*)], and air-dried chromosome preparations were made by the method of Moorhead *et al.* (*4*) on quartz slides. All of the metaphases examined contained the altered chromosome characteristic of chronic granulocytic leukemia, the Ph^1 chromosome, but there were no other demonstrable abnormalities.

Slides known, from a preliminary survey with a phase microscope, to have good metaphase figures were treated with ribonuclease (*5*) and mounted in glycerine under a quartz cover slip. The principal chromosomal substance absorbing at 257 mμ is expected to be DNA. We measured the integrated or "total" absorbance (A_T) of the chromosomes by densitometric scanning of photomicrographs taken with the cadmium emission line at 257 mμ (*6*).

Table 1. Integrated absorbance (A_T) at 257 mμ of human metaphase chromosomes in leukemic cells from an individual with chronic granulocytic leukemia. Ribonuclease treatment of the chromosomes (*5*) renders the A_T values proportional to DNA content (but see text). The numbers of homologs (or members of the group 21–22) measured in each nucleus are entered in parentheses after the mean value for a member of the group in each of eleven nuclei. The last column lists the mean length of the chromosome(s) 1 in each nucleus measured with a map measure from photographic enlargements.

Chromosome group			Length No. 1
1	21–22	Ph^1	(μ)
Diploid cells			
1.232(1)	.247(3)	.139(1)	7.6
0.968(2)	.159(2)	.127(1)	8.4
0.825(2)	.176(3)	.111(1)	9.9
0.777(1)	.169(3)	.109(1)	9.9
0.867(2)	.157(3)	.115(1)	8.8
0.892(2)	.168(3)	.092(1)	7.7
0.591(1)	.147(3)	.097(1)	9.9
0.883(2)	.161(3)	.130(1)	7.4
Tetraploid cells			
0.521(1)	.151(3)	.056(1)	11.8
0.681(2)	.126(3)	.076(2)	8.2
0.603(1)	.151(1)	.077(1)	8.7
Mean ± standard error of mean			
0.804 ±0.061	0.165 ±0.009	0.103 ±0.008	8.9 ±0.4

This report (*7*) is based on 11 nuclei in which the Ph^1 chromosome, at least one of the 21–22 group and a chromosome number 1 were measurable. Other chromosomes that were cytologically identified, insofar as they were definitely assignable to groups in the Denver classification (*1*; see also *8*), also were measured when half or more of the chromosomes in a group was technically suitable for measurement. Eight of the nuclei were from diploid cells, that is, 46 doubled chromosomes were displayed on the metaphase plate; three of the nuclei were from tetraploid cells (92 doubled chromosomes). Mitosis had been arrested at metaphase by the colchicine treatment. Seven nuclei were from one slide, four from another.

The numerical results for chromosomes 1, 21–22, and Ph^1 are given in Table 1. Chromosomes 21 and 22 were not morphologically distinguishable from each other in these preparations, and the differences between them in a nucleus were well within the errors of the method; the values for the group in each nucleus were combined to obtain the arithmetic means entered at the bottom of Table 1. The ratio of the mean Ph^1 to mean 21–22 in Table 1 is found to be 0.62. Because the individual A_T values were spread over a twofold range and because it is the relative DNA content of the two chromosomes that is of interest, statistical treatment was carried out on logarithms of the individual entries. The mean difference between the $\log_{10}$ Ph^1 and $\log_{10}$ 21–22 of the absorbancies was 0.2137 with a standard error of the mean of ±0.0291, which corresponds to a geometric mean for the ratio of Ph^1 to 21–22 of 0.61 ± 0.01.

In the face of the wide range of values for the A_T of a chromosome type in different nuclei it was necessary to test for the possibility that the ratios of the DNA contents of the different chromosomes were different in different nuclei. That was accomplished by a variance analysis on the differences between the logarithms of the entries in Table 1; the differences between nuclei with respect to the ratio of one chromosome to another were found not to be significant ($P > 0.2$). It may be concluded that the Ph^1 chromosomes had the same relative DNA content in all of the nuclei measured, a conclusion compatible with the supposition that all are derived from a common ancestral chromosome in this individual.

The origin of the Ph^1 chromosome, though still obscure, can be thought of as a deficiency or as a simple reciprocal translocation between 21 and another chromosome as outlined. If a translocation involved any of the small chromosomes (numbered 16 or higher), then the partner with which number 21 had exchanged would have been detected in the detailed morphological studies of Nowell and Hungerford (*2*). If one of the large chromosomes (numbered 5 or lower) were involved, then the change in one homolog engendered by the hypothetical translocation would be very difficult to see in the microscope (*8*), but the translocated homolog could be de-

tected by absorption measurements, if the precision of the measurements were sufficiently high. The data necessary to evaluate the precision, namely the A_T of the appropriate chromosomes and the standard deviation of a measurement of a homolog, were determined for our material. The standard deviation, based on the differences between 28 pairs of homologs within nuclei, is ± 0.059 units of A_T and is not demonstrably dependent on chromosome size. Since the translocated piece would have about the same absorbance (0.062 units from the means in Table 1) it is obvious that we could not detect it, if it did exist, by a comparison of the two homologs in a single nucleus. An alternative procedure to detect the translocation, that is, a comparison of the variability between homologs in many nuclei for different chromosomes, would not be practical by so laborious a method as the photographic one. That direct electronic measurement would also be inadequate is clear from the variability reported by Carlson *et al.* (*9*) in a recent application to mammalian (Chinese hamster) chromosomes.

It must be mentioned in passing that the highly significant differences between nuclei (below the 1 percent level of probability) with respect to A_T of individual chromosomes (Table 1) or whole nuclei (Table 2) cannot be attributed to the pathological condition of the individual from whom the material was taken. A separate analysis of the lengths in Table 1 (*10*) shows that low values of A_T are associated with the most extreme effects of treating the cells with a hypotonic solution (hypotonic serum in this case) before fixation. This prior treatment tends to uncoil the metaphase chromosomes rendering them morphologically more distinctive and longer (*11*); the correlation coefficient (r) between A_T and length of chromosomes 1 in Table 1 is significantly different from zero ($r = -0.62 \pm 0.25$). This source of variability does not affect the comparisons we have made within nuclei; it does, however, vitiate any conclusions that might be drawn concerning the relation between the content of DNA of chromosomes in diploid and tetraploid nuclei (Table 1) (*12*).

The magnitude of the amount of DNA lost (as in a deficiency) or translocated in a chromosomal rearrangement to produce the Ph^1 chromosome can be estimated either directly from the microspectrophotometric data or from the relative absorbance values and the chemically determined average DNA in a nucleus. Conversion of absorbance to mass of DNA requires knowledge of the specific absorptivity of DNA as it exists in these chromosomes, a parameter which is not precisely known. As a first approximation we have used a typical value determined by measurement in dilute solution (*13*), and calculated to be 2.6 units of A_T for one picogram of DNA per square micron (*14*). The sums of the A_T values in the five nuclei in Table 2 are 28.54, 24.37, 20.55, 19.70, and 19.0, respectively, from which we obtain 10.9, 9.4, 7.9, 7.6, and 7.3 pg of DNA in the five metaphase plates. These can be compared with chemically determined values such as those of Davidson *et al.* (*15*): 7.3 pg in leukocytes, 8.6 pg in kidney, 8.7 pg in bone marrow, and 10.4 pg in liver, all expressed per nucleus. The chemical determinations, it should be remembered, are expected to be high because of the presence of nuclei with more than the "diploid" amount of DNA in most somatic tissues (*16*) while the values we have determined are for complete metaphase nuclei, that is, the "tetraploid" amount. The "deficiency" in a CGL karyotype, according to the chemical data, would be 0.27 percent of a nucleus (Table 2) with 7.3 pg of DNA, or 0.02 pg, equivalent to 2×10^7 nucleotide pairs. Direct conversion of half the difference between the mean A_T values determined by microspectrophotometry for 21–22 and Ph^1 in Table 1 gives 0.012 pg of DNA or about 10^7 nucleotide pairs. The range of values in Table 1 would be 0.006 to 0.02 pg or 0.6 to 2.0×10^7 nucleotide pairs per diploid chromosome set.

Table 2. Integrated absorbance (A_T) at 257 m_μ of groups of human chromosomes (Denver classification) in metaphase plates of diploid cells from a female with chronic granulocytic leukemia. The numbers in columns 2–6 are the total A_T for all of the chromosomes in the group based on measurement of the number of chromosomes given in parentheses. The total number of chromosomes measured out of the possible 46 is given in the last line. Column 7 shows the mean percentage of total nuclear A_T found in each group in a diploid nucleus, and its standard error, based on the individual percentages in the five nuclei. See legend to Table 1 for further explanation.

Denver group*	Nucleus code number					Mean % of nucleus
	17-4	8-8	17-9	17-5	17-8	
1	2.46(1)	1.94(2)	1.65(2)	1.55(1)	1.73(2)	8.33±.24
2	2.10(2)	1.74(1)	1.67(2)	1.56(2)	1.42(2)	7.57±.18
3	1.81(2)	1.67(1)	1.26(1)	1.26(2)	1.36(2)	6.56±.19
4-5	3.32(4)	3.01(3)	2.04(3)	2.21(3)	2.35(4)	11.53±.45
6-X-12	11.21(15)	9.82(14)	8.20(15)	7.65(12)	7.05(16)	39.17±.56
13-15	3.03(6)	2.48(5)	2.22(4)	2.17(4)	2.00(6)	10.61±.15
16	0.85(2)	0.63(2)	0.59(2)	0.51(1)	0.55(2)	2.79±.12
17-18	1.66(4)	1.32(4)	1.17(4)	1.30(3)	1.13(3)	5.87±.20
19-20	1.22(4)	1.17(4)	1.10(1)	0.87(2)	0.82(3)	4.62±.20
21-22	0.74(3)	0.48(2)	0.53(3)	0.51(3)	0.47(3)	2.43±.12
Ph^1	0.14(1)	0.13(1)	0.11(1)	0.11(1)	0.12(1)	0.54±.02
Number	(44)	(39)	(38)	(34)	(44)	

*See reference (*1*).

The question of whether the CGL karyotype is deficient for a part of chromosome 21 or whether the Ph^1 chromosome is a visible manifestation of more subtle interchromosomal changes remains unresolved by our data. The most serious impediment to further investigation along these lines lies now in the preparation of the material, which so far appears to introduce variations in content of DNA far larger than the cytogenetic ones sought, and which must be investigated before microspectrophotometric data can be more effectively interpreted.

GEORGE T. RUDKIN
DAVID A. HUNGERFORD
Institute for Cancer Research, Fox Chase, Philadelphia 11, Pennsylvania
PETER C. NOWELL
Department of Pathology, School of Medicine, University of Pennsylvania Philadelphia 4

References and Notes

1. We designate human chromosomes by the Denver system in which the autosomes are numbered consecutively from 1, the longest, to 22, the shortest. "A proposed standard system of nomenclature of human mitotic chromosomes" has appeared in *Lancet* **1960-I**, 1063 (1960); *Am. J. Human Genet.* **12**, 384 (1960); *J. Am. Med. Assoc.* **174**, 159 (1960) and elsewhere. See also the account of the more recent London conference on the normal human karyotype in *Cytogenetics* **2**, 264 (1963).
2. P. C. Nowell and D. A. Hungerford, *J. Natl. Cancer Inst.* **27**, 1013 (1961).
3. P. C. Nowell, *Cancer Res.* **20**, 462 (1960).
4. P. S. Moorhead, P. C. Nowell, W. J. Mellman, D. M. Battips, D. A. Hungerford, *Exptl. Cell Res.* **20**, 613 (1960).
5. Crystalline, salt-free ribonuclease, Worthington, 1 percent, *p*H 7.0, 2 hours, 37°C.

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 nonspecific absorptivity of the protein (for example, serum proteins) is 0.1 to 0.01 that of DNA at 257 mμ, 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 mμ 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.

17 January 1964

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.

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. 2*a*) 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. 3*a*). 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