were formed. These later produced small roots at one end (Fig. 2), and, at the other end, typical leafy structures gradually developed so that normal plantlets were formed.

Different stages of embryoid formation from single cells are shown in Fig. 1, *A* to *E*, and various stages of growth and differentiation of the embryoids are shown in Fig. 2. Although not proved, the plantlets probably arose from single cells alone, as did the carrot plants grown previously (*1*, *2*). We repeated our experiments several times. Differentiation and plantlet formation were achieved equally well even when the tissue had been subcultured in liquid MS-medium up to three times.

This rules out the possibility that residual growth substances of coconut milk were accidentally transferred to the MS-medium along with the tissue inoculant isolated from the C-medium. If the callus explants taken from the stock cultures on the C-medium were not growing vigorously, differentiation and organ formation in the liquid MS-medium often stopped after the embryoids or embryoids with roots had developed.

Attempts to obtain mature adult plants from the plantlets by growing them on filter paper, sand, or vermiculite moistened with MS-medium, Hoagland's salt solution, and water, have failed. When the plantlets were removed from the liquid or the agar medium and placed on fresh medium with or without agar, the entire root surface formed callus tissue from which new roots developed.

The growth of the tissue suspended in the liquid medium was so fast (6.045 g of tissue could be harvested after 5 weeks of growth) that the medium was soon filled with cells, cell groups, embryoids and plantlets. The medium, originally clear, became chalky white in color toward the end of the growth period. This was partly due to the thick suspension of cells and cell fragments resulting from dying or dead cells, and also to the presence of an opaque, fibrillar material (shown in the background of Fig. 1, *C–E*) which may be an excretory product of the cells.

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5. In similar experiments with tissues derived from the petiole and stem of *Lactuca sativa* (lettuce), roots are often formed from the callus tissue. With tissue from *Petro selinum hortense* (parsley), roots are easily induced by keeping the cultures in the dark (when grown on agar) and by supplying low amounts of adenine; in one instance a shoot with typical curled green leaves and roots has developed in MS-medium with adenine. Results of these experiments will be published in detail elsewhere.
6. T. Murashige and F. Skoog, *Physiol. Plantarum* **15**, 473 (1962). To the medium used in this study we added the following supplements to the inorganic salt solution (mg/liter): thiamin hydrochloride, 0.1; nicotinic acid, 0.5; pyridoxine hydrochloride, 0.5; glycine, 2; myo-inositol, 100; indoleacetic acid, 10; kinetin, 0.04. Sucrose was added at the rate of 3 percent, and agar, when used, at the rate of 1 percent.
7. Supported in part by the U.S. Army Natick Laboratories, Natick, Mass., contract DA19-129-QM-1817 (01 5123), by the American Cancer Society, by NIH, and by the Research Committee of the Graduate School from funds supplied by the Wisconsin Alumni Research Foundation.

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Temperature Dependence of the Activity of the Antitumor Factor in the Common Clam

Abstract. *The tumor inhibiting action of extracts of the common clam,* Mercenaria mercenarius, *is very low in the winter and can be restored to the high summer values by heat.*

Extracts prepared from various tissues (*1*) and urine (*2*) were found in this laboratory to contain two factors, one which inhibits the growth of tumors and another which promotes tumor growth. The presence of two antagonistic substances made the isolation of the active principles difficult. Schmeer (*3*) found that watery extracts of the common edible quahog possess a similar inhibitory action without containing the promotor, or containing the promotor only in relatively small amounts. We confirmed these results on Krebs-2 tumors in mice. One-third of the activity was found in the fluid which occurs between the body of the clam and valves, and two-thirds, in extracts of the body. Both the fluid and extracts of the body were toxic and required purification before they could be tested in vivo for their action on tumor growth. In the experiments that are described here, which were started in July 1963 and continued until December of the same year, only watery extracts of the body were used.

The body was removed from the valves and was minced in a Waring blender and extracted with 2 volumes of distilled water. The extract was centrifuged at 8000 rev/min for 10 minutes and the supernatant was concentrated with Aquacide (*4*) to one-tenth of its volume. The concentrated extract was dialyzed against distilled water with constant stirring in the refrigerator and was then lyophilized. From 1 kg of clam body 18 to 20 g of lyophilized product were obtained, the activity of which varied according to the temperature. In Table 1 the activity of the extracts is expressed in retine units (*2*) and calculated for 1 kg of clam body (wet weight).

As shown in Table 1, the activity of the extracts dropped in the winter to very low values. To find out whether this was merely an effect of the temperature and whether the higher activity could be restored by increasing the temperature, about 40 to 50 clams were placed in a basin (360 by 90 by 32 cm) and were subjected to a flow of sea water, 10 to 14 liters per minute. The temperature of the water was varied from 5° to 21°C. After 1 to 4 weeks in the basin the clams were removed and extracts were prepared as before. The activity of these extracts is shown in Table 2.

These experiments indicate that the activity of the antitumor factor in clams depends on temperature, and that

Table 1. Activity of clam extracts obtained at various times during 1963.

Date extract obtained	Activity (retine units/kg)
5 July	1600–1800
8 July	1600–1800
15 July	1600–1800
24 July	2000–2400
1 Aug.	2500–2800
7 Aug.	2800–3000
15 Aug.	2800–3000
20 Aug.	2800–3000
24 Aug.	2800–3000
5 Nov.	600– 700
12 Dec.	350– 400
10 Feb.	346

Table 2. Activity of extracts of clams kept in a bath with a flow of water maintained at different temperatures. Before being placed in the bath the clams had been kept in an outdoor pool, approximate temperature 5°C, for 90 days. The activity of extracts obtained from clams after removal from the pool was 346 units.

Temperature of bath (°C)	Time in bath (days)	Activity (retine units/kg)
15.5	7	300–500
21.0	7	400–500
15.5	14	1355
15.5	14	1122
21.0	14	1400
15.5	26	2981
15.5	47	2900–3000

the high summer values can be restored by keeping the clams in warm water for 4 weeks before preparing the extracts.

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Chromosome Complements of Two Species of Primates: Cynopithecus niger and Presbytis entellus

Abstract. *Forty-two chromosomes were counted from expanded cultured cells of four Celebes apes,* Cynopithecus niger. *Mitotic cells from three Indian langurs,* Presbytis entellus, *contained 44 chromosomes. These counts support cytogenetically the taxonomical classification of these animals according to Simpson.*

At least part of the confusion that still attends the classification of primates has been created by those students "who had no experience in taxonomy and who were completely incompetent to enter this field" (*1*). New species have been commonly named entirely on the basis of gross morphology, coat color-pattern, or the geographical location of a single specimen. Modern classification, however, is based on representatives of a population and on the integration of social behavior, ecology, geography, gross anatomy and physiology, and paleontology; species designation based upon such broad biological lines has greater validity. In recent years blood typing (*2*), immunologic properties (*3*), and chromosome analysis (*4*, *5*) have also become indispensable criteria for animal classification.

In this report we present the chromosome counts of two species of primates from the colony of the Oregon Regional Primate Research Center. This information supports the present classification according to Simpson's taxonomic scheme.

Tissues from four specimens of *Cynopithecus niger* (two males and two females) were examined. Monolayer cultures of kidneys and lungs were grown in vitro (Melnick, *6*). Bone marrow taken from the femur with a heparinized syringe was dispensed into a flask containing medium 109 (*7*) and 15 percent fetal calf serum and incubated until there was a sufficient growth of cells for chromosome preparations. The metaphasic cells were expanded according to the methods of Tjio and Puck (*8*) and Rothfels and Siminovitch (*9*).

Ninety percent of well-expanded chromosomes of the cells prepared from bone marrow, kidney, and lung cultures exhibited a diploid number $2n = 42$ (Fig. 1*A*). Analysis of chromosomes based on the centromere position (*10*) showed 11 pairs of metacentric and 9 pairs of subterminal chromosomes. Of the sex chromosomes, the X chromosome was subterminal and the Y chromosome appeared acrocentric. The satellite pair was considered to be in the metacentric group and was generally the fourth to fifth longest.

These animals, commonly called Celebes apes, are found in the Celebes Islands and several other islands east of Wallace's Line. In general, fauna east of the Line has been more isolated from continental Asia than that west of the Line. Male and female adult *Cynopithecus* have a characteristic crest of hair at the top of the head and darkly pigmented skin, and are covered with heavy, black body-hair. Although tailless, the body-build, facial architecture, and locomotor pattern of these animals place them closer to the macaques than to any other form of primate in Southeast Asia.

As further evidence of this relationship, the chromosome number of 42 is the same for *Cynopithecus* and all known *Macaca* (*9*, *11*), although the centromeres are in somewhat different positions. The African counterpart of these animals, the baboon (*Papio*), also has 42 chromosomes, with ten pairs each of metacentric and subterminal autosomes, and X and Y metacentric sex chromosomes (*11*). Our karyograms for two male Guinea baboons are in close agreement with those previously described by Chu and Giles (*11*), except that we considered the Y chromosomes to be acrocentric rather than metacentric. The satellite pair was considered metacentric and was about the fifth largest in that group. These fundamental cytological similarities support Simpson's classification of the Celebes ape in the subfamily Cercopithecinae with the macaque and baboon.

We also examined cultured tissues from the kidneys and bone marrow of three Indian langurs (*Presbytis entellus*). At least 90 percent of the countable metaphasic cells examined had a diploid number of 44 chromosomes (Fig. 1*B*). There were 14 metacentric and six subterminal pairs and one acrocentric pair of autosomes, and subterminal X and Y chromosomes. The satellite pair was considered metacentric and was nearly always the smallest in size. The count of 44 chromosomes in our specimens does not agree with that of Makino (*12*), who reported 50. Although Makino studied cells from only one animal, the discrepancy in these findings may be attributed to today's improved techniques. For another species of Colobinae from West Africa, *Colobus polykomos*, Bender and Chu (*4*) reported a count of 44 chromosomes, with 28 metacentric, 14 subterminal, 1 metacentric X, and 1 acrocentric Y. One of the cytogenetic differences noted between these two species is the position of the male sex chromosome.

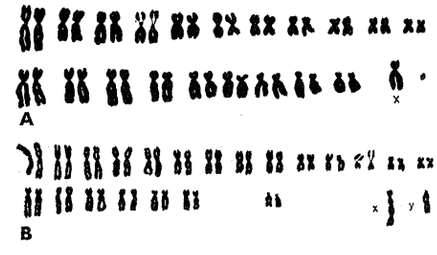

Fig. 1. (*A*) Representative karyotype of a male *Cynopithecus niger*. From a cultured bone marrow cell with a count of 42 chromosomes. (*B*) Representative karyotype of a male *Presbytis entellus*. From a cultured kidney cell with a count of 44 chromosomes.