concentration of superoxide dismutase in heart muscle (183 µg equivalents/g wet tissue weight) was twice that in each of the 5 skeletal muscles studied (53–88 µg equivalents/g wet tissue weight). The SOD activity of most muscles did not change after starvation and refeeding or between 12 and 15 months of age. Thus, while the functions of catalase and SOD are closely related, starvation did not affect them in the same manner.

The significance of the increase in catalase activities after starvation is not clear. Electron micrographs of selected muscles after starvation revealed no gross degeneration or disturbance of contractile proteins (unpublished observation). Catalase did not correlate with the percent decrease in muscle weight (table). Therefore, the use of catalase as a quantitative indicator of muscle weight loss is questionable. Mitochondria in skeletal muscles show extensive hypertrophy and elongation and an increase in number with fasting[9]. These changes were apparent as early as the 1st day of starvation and the alterations in muscle morphology disappeared shortly after refeeding a normal diet. The soleus is a muscle with more mitochondria and a higher oxidative capacity than white muscle[10]. This muscle also has a higher catalase level than white muscles, such as the EDL. Therefore, one is tempted to speculate that the increase in catalase with starvation is related to a possible increase in the number of mitochondria.

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Mitotic response and sister chromatid exchanges in lymphocytes cultured in sera from different sources[1]

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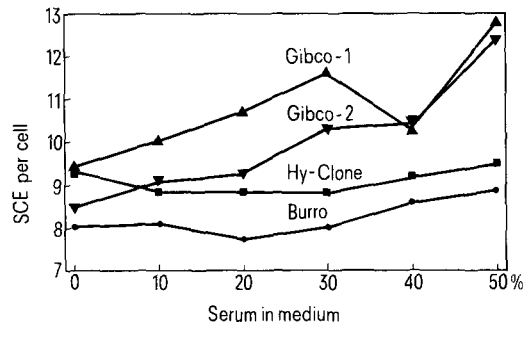
Summary. The numbers of sister chromatid exchanges in lymphocytes grown in varying concentrations of serum from different sources indicated that some sera contain a factor, probably introduced as a contaminant, which induces SCEs. Sera from 6 animals showed no evidence of a difference in baseline SCE levels due to the donor of the serum.

Sister chromatid exchanges (SCE) scored in cultured cells are recognized as a sensitive measure of the mutagenic action of various chemicals in mammalian systems (see review of Latt et al.[2]). The overall sensitivity of the system is almost certainly reduced by the many variables associated with the in vitro growth of cells, not the least of which is variation in composition of the growth medium. Identification of some of the contributing variables would help reduce the range of baseline SCE rates which are reported to vary, for example, from 6.9 to 15.1 per cell in human lymphocytes[3,4]. Fetal calf serum, which is almost universally used as a media component, has been blamed by Kato and Sandberg[5] for inducing some of the SCE seen in control cultures. They noted differences in SCE rates among Chinese hamster cells grown in media containing sera from different commercial sources; the rates were sharply reduced by heat inactivation of the sera. Although differences in collection and handling processes could obviously influence the SCE-inducing properties of sera, inherent differences in the sera of different donors could also contribute. Chatot et al.[6] recently noted that the growth of rat embryos cultured on sera from different donors varied significantly more than that of embryos grown on serum from a single donor.

In this report we present further data on sera from different sources which suggest that variations in processing procedures are of primary importance in determining the SCE-inducing potential of serum.

Materials and methods. Samples of serum collected at our laboratory from 6 adult burros (3 male, 3 female) were tested to see whether sera from different animals would vary in their ability to induce SCE. Lymphocytes from 2 human and 2 pig donors were simultaneously grown in media made with sera from each of the 6 animals. Sera used for the dose-response comparisons were 2 lots of fetal calf serum from Gibco (Grand Island, N.Y.), 1 lot of Hy-Clone fetal calf serum (Sterile Systems, Inc., Logan, Utah)



Sister chromatid exchanges in swine lymphocytes grown in media containing increasing concentrations of serum from different sources. Points are means of 25 cells from each of 2 animals.

Table 1. Sister chromatid exchanges in lymphocytes cultured in media containing sera from different animals

Source of serum	Source of lymphocytes tested				Average
	Human		Pig		
	1	2	1	2	
Burro 1 ♂	11.40±0.84*	8.56±0.74	11.12±0.86	10.60±0.89	10.42±0.43
2 ♂	11.96±0.95	10.04±0.71	11.88±0.68	11.24±0.89	11.28±0.41
3 ♂	11.92±0.90	10.80±0.85	11.36±0.76	9.48±0.66	10.89±0.40
4 ♀	11.48±1.01	9.36±0.81	11.24±0.91	11.12±1.15	10.80±0.49
5 ♀	10.88±0.70	9.44±0.54	9.80±0.66	10.08±0.72	10.05±0.33
6 ♀	11.92±0.66	8.64±0.55	11.56±0.58	10.00±0.64	10.53±0.33
Average	11.59±0.34	9.47±0.29	11.16±0.31	10.42±0.34	10.66±0.29

* Mean ± SE for 25 2nd-division metaphases.

Table 2. Sister chromatid exchanges among swine lymphocytes cultured in media containing various concentrations of serum

% serum in medium	SCE/cell
0	8.81±0.25*
10	9.01±0.26
20	9.13±0.27
30	9.69±0.31
40	9.62±0.28
50	10.88±0.38

* Mean ± SE for 200 metaphases.

Table 3. Percent of swine lymphocytes reaching second or third metaphase during 50 hours of culture in media made with different sera

% serum in medium	Source of serum			
	Gibco-1	Gibco-2	Hy-Clone	Burro
0	90.0*	88.0	86.3	80.3
10	93.1	89.8	90.6	81.0
20	90.1	91.3	84.0	79.8
30	83.1	77.5	66.8	73.3
40	47.8	54.8	54.3	58.1
50	43.5	43.6	36.4	53.3

* Mean for 200 metaphases from each of 2 animals.

and a composite of samples from a 2nd group of 6 burros. Burro blood was aseptically drawn from healthy animals and allowed to stand at room temperature until a clot had formed and retracted (4 h); serum was then drawn off and centrifuged at 500 × g to remove any cellular material. All sera were heat inactivated at 56 °C for 30 min, filter sterilized, and stored at 5 °C until used (within 3 days). Completed media contained TC-199, 1% phytohemagglutinin-M, 200 units penicillin and 200 µg streptomycin per ml, and the appropriate concentration of serum. Cell-rich plasma was obtained from samples of human and swine blood by sedimentation and/or centrifugation and used to inoculate cultures. The quantity of inoculum was adjusted to yield 10^6 lymphocytes/ml for swine and 0.5×10^6/ml for humans in the final culture (0.27–0.50 ml of inoculum per 10 ml medium was required for wine andd 0.41–0.82 for humans). After 24 h, 5-bromodeoxyuridine (BrdUrd) was added at a final concentration of 5 µg/ml of culture; cells were incubated in the dark at 37 °C.

Swine cells were harvested after 50 h in culture and human cells after 56 h; colchicine was present for the final 2 h in all cases. Air-dried preparations were stained by the FPG method of Perry and Wolff[7] and 25 2nd-division metaphases from each culture were scored for SCE. The rates at which cells divided were compared by scoring 200 randomly-selected metaphases as being in their 1st, 2nd or 3rd in vitro division based on the differential staining patterns among their chromosomes.

Results. Table 1 shows the similarities in the numbers of SCE found among lymphocytes cultured in media containing 20% serum from different adult burros. There were no statistically significant differences among the sera from the 6 animals although a difference ($p < 0.05$) was noted in the overall sensitivity of lymphocytes from the different donors. The proportion of cells which had reached either 2nd or 3rd metaphase during the culture period did not vary appreciably between these sera; among pig cells 72–79% fell in this category while human samples varied from 48 to 58%.

The increase in SCE per cell resulting from increasing the serum concentration in media is averaged for the 4 sera in table 2. Although the overall mean for the 4 sera increased with increasing concentration, the dose-response curves of the figure indicate that this association was attributable almost entirely to the effects of 2 of the sera. Cells in the burro and Hy-Clone sera showed very little effect of increasing the amount of serum in their media; however, both lots of serum from Gibco produced a general increase in SCE in relation to the increasing serum concentration. Analysis of variance of these data indicated a significant difference among the sera ($p < 0.05$) and an effect due to serum concentration which was very nearly significant. The concentration effect and the serum X concentration interaction were further refined by a linear models procedure which tests the relative contributions of linear, quadratic and cubic relationships. An overall linear relationship between serum concentration and SCE numbers was indicated by the fact that only the linear component was significant. A significant difference in magnitude between the regression lines for the 4 sera was also noted; however, the linear X sera component, which compares slopes of the regression lines for the different sera, closely approached but did not attain significance. The sensitivity of this latter comparison is limited somewhat by the fact that lymphocytes from only 2 animals were tested in each serum.

The proportion of cells which attain their 2nd or 3rd metaphase during a constant culture period provides a measure of the responsiveness of cells to different culture conditions and gives an indication of the cytotoxic effects of any material added to the medium. The suppressive effects of high concentrations of all the sera on cell division are evident in the data or table 3. Maximum division rates were attained by cells in media containing 10–20% of the different sera. Swine lymphocytes respond quite well under a variety of in vitro culture conditions as evidenced by the fact that total elimination of serum from the medium caused only a slight reduction in their mitotic rates. The maximum division rate was somewhat lower in burro serum than in the fetal calf sera although burro serum

showed less cytotoxic effect at the higher concentrations. It is uncertain whether the slightly lower mitotic rate in burro serum indicated a change in the cell cycle or simply a lengthened response time to the mitogen; however, we do not have any evidence that it influenced the SCE dose-response curves.

Discussion. Although serum of fetal calf origin is most commonly used in cell culture media, intuitively we would expect serum from adult animals to show a greater variability of composition and of SCE-inducing factors if they exist. The intent of testing sera from different burros was thus to use adult animals in order to maximize the likelihood of detecting differences. The similarity of SCE numbers in cells grown in sera from the different animals indicated that the inducing factor either occurs quite uniformly among individuals or is not prevalent at all in burro serum. Any contribution by factors carried into the cultures by the lymphocyte donor's serum would have been minor because of the relatively low volumes of inoculum used and would have been neutralized by the fact that the same volume of a donor's serum was added to each of the test cultures.

If serum contains factors that induce sister chromatid exchanges, then the number produced should be related to the concentration of serum to which the cells are exposed. Such a relationship is quite obvious for both lots of the Gibco serum used in this study, whereas the Hy-Clone and burro sera produced only slight increases at high concentrations. This situation suggests that while some commercially available sera do in fact contain a factor that causes SCE, this factor is not only absent from burro serum but probably occurs in these fetal calf sera as a contaminant rather than a normal constituent. Varying levels of contamination could have accounted for the difference in SCE induction by the sera tested by Kato and Sandberg[5], even after their heat inactivation and dialysis treatment had reduced the overall activity level. While our data provide no measure of a possible reduction in activity of the factor by heat treatment, it is obvious that the heat inactivation to which all our sera were treated did not eliminate the agent.

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Genes regulating the appearance of two kinds of fruit in *Microseris* strain B 87 (Asteraceae: Compositae)[1]

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Summary. The annual hybrid strain *Microseris* B87 *(M. pygmaea* × *M. bigelovii)* produces 2 kinds of fruit (achenes) on its flowering heads, outer hairy and inner smooth ones. 2 unlinked genes are identified that determine the relative number of hairy achenes, on a logarithmic scale.

The composite flowering heads of the Asteraceae offer unique possibilities for the genetical analysis of developmental control processes. Both the bractlike phyllaries surrounding the capitula, and the florets, arise from a continuous spirally arranged series of primordia. The production of these primordia can be regarded as a linear sequence in time. Genetic regulating mechanisms determine the kind of organ that arises at each homologous site. These regulating mechanisms appear to be irreversible switches. The spiral arrangement of the primordia on a dome-shaped head causes the typical appearance of a concentric arrangement of the various organs with the earlier ones (e.g. phyllaries) on the outside and the later ones (e.g. disk florets) on the inside. Any delay in the timing of the developmental switch will result in a broader ring of outer structures at the expense of inner structures. Variation of this kind is very common both in horticultural varieties of cultivated Asteraceae and in natural populations. We have begun using this effect in order to detect genes regulating development in the lactucean genus *Microseris.*

In the small annual species of the section *Microseris,* there is usually a rather clear distinction between outer florets giving rise to outer achenes and inner florets giving rise to inner achenes[3-5]. This distinction involves a series of characters. One of these, the hairy or furry appearance of the outer achenes as opposed to smooth inner achenes, is very easy to score in all achenes on a head, including sterile ones[4,5]. There are plants with no hairy achenes ever, plants with nothing but hairy achenes, and a whole series of intermediate types. Our preliminary estimate of at least 10 genotypes contributing to this variability[5] could mean that there are about 3 genes involved in the determination of the timing of the transition from hairy to smooth achenes in addition to genes that are responsible for the expression of the hairy character. Thus a complete analysis of the 'hairy achenes' polygenic system appeares to be feasible. Here we report the first results of the genetic analysis of the hybird strain B87. The exact strain designation is important because each of our annual strains is obtained by spontaneous selfings from one single ancestral plant. Each strain therefore represents a minute selection from the vast pool of genetic variation present in the genus, and the genetics of conspecific strains or of repeated hybridizations of the same taxa may be very different. Strain B87 consists of segregants from a single hybrid specimen between *M. pygmaea* (Chambers accession 246: few, weakly hairy, outer achenes, the inner achenes bearing a tuft of hairs on top) and *M. bigelovii* (Chambers accession 207: about 30% strongly furry outer achenes, the inner achenes without hairs either on the sides or on top)[6]. The F1 and a preliminary small F2 family of 10 plants, 8 of which gave rise to