tery. However the rise was no more prompt than that of SGOT and occurred later than ECG changes. Extent of the Mn elevation did not correlate with size of the infarct. No difference in Mn content was observed between normal and infarcted areas of myocardium. In human patients, serum Mn was not always elevated following myocardial infarction and was also increased in other diseases. Normal values for the dogs averaged 3.7 μg%, for the humans, 3.9.

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Quantitative Effects of Alkyl Benzene Sulfonate (ABS) on KB Cells in Tissue Culture.* (29372)

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During the past few years synthetic detergents have displaced soap as the cleanser of choice, both domestically and industrially. The most widely used are those containing as a major constituent sodium dodecyl benzene sulfonate (ABS), an anionic surfactant. Whereas soap is easily degraded by natural microbial activity, the synthetic detergents have proven almost completely refractory. As a result, they appear to be present to a considerable degree in ground water or as residues on inadequately rinsed utensils and cutlery. This has led to the suggestion that a potential hazard may occur from such consumption.

Classical toxicological techniques for evaluating efficacy of chemicals for human consumption are time-consuming and extremely costly. Methods that may signal untoward metabolic reactions in a short period could be extremely valuable as a screening device to indicate the need for additional testing by established pharmacological methods of those chemicals that exhibit cytopathological lesions in *in vitro* tests. Tissue culture may offer just such an accelerated, relatively inexpensive procedure.

The present study was undertaken to ascertain several measurable physiological responses in an established cell line to graded doses of ABS. For this purpose we have used KB cells which were originally isolated by Eagle(1) from a human epidermoid carcinoma.

Materials and methods. A replicate series of KB cell cultures was set up by pipetting 10 ml from a large cell suspension into 150 ml milk dilution bottles. A nutrient mixture

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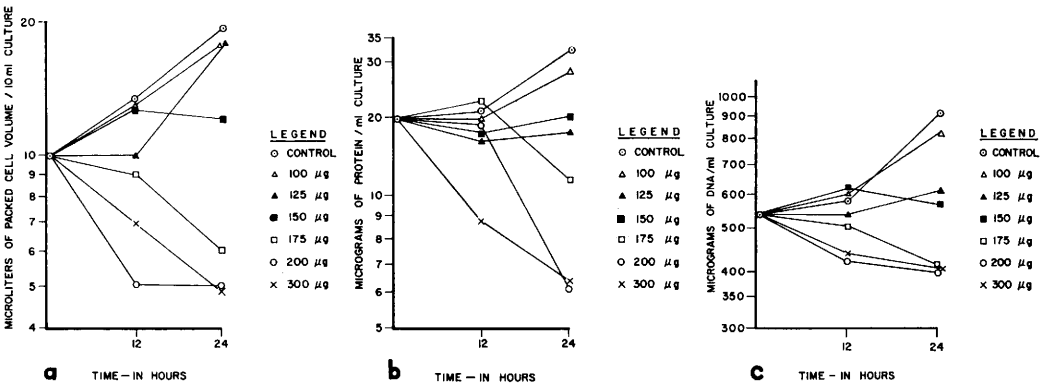

FIG. 1. Effect of varying concentrations of purified ABS cn physiological response of KB cells. (a) µl of packed cells. (b) µg of protein. (c) µg of DNA.

supplemented with 10% calf serum constituted the growth medium(1). These bottles were incubated at 37°C for monolayer formation.

The time when the cell cultures were in the logarithmic growth phase was determined from the increases in packed cell volume (P.C.V.). At that point, the spent growth medium was decanted and replaced by fresh medium containing graded concentrations of ABS.

The following series of experiments, modified only by the number of replicate cultures, were carried out using this procedure. Crude or commercial ABS contains a variety of ancillary chemicals added to enhance its detergency. To remove these materials, which might affect physiological responses, the crude material was extracted with ethanol. Refluxing filtration and drying obtained a purified compound.

1. Purified ABS, at final concentrations of 300, 200, 175, 150, 125, 100 and 0 γ per ml, in the growth medium was added to bottles, in triplicate. Samples were taken for analysis after 12 and 24 hours.

2. Each day fresh medium containing 100 γ/ml replaced the spent medium. An appropriate control series was included. ABS-free medium was treated similarly. Packed cell volume, DNA and protein determinations were made after one week's incubation.

3. Purified ABS, at an initial concentration of 20 γ/ml, was added to a third series. Each day a 20 γ/ml increment of ABS was added in fresh medium after decanting the previous day's material. Appppropriate controls were included.

4. Crude ABS at final concentrations of 100 and 10 γ/ml in growth medium was added to a fourth series.

During the test period, cell development was followed in each culture by analysis of packed cell volumes and synthesis of deoxyribonucleic acid (DNA) and protein as previously reported(2).

Results and discussion. The detergent appears to evoke an inhibitory response on cell growth at all concentrations tested (Fig. 1a, 1b, 1c). A direct relationship between detergent concentration and degree of inhibition is suggested. Above 175 γ/ml a striking decrease in PCV, DNA and protein synthesis was noted which could be correlated morphologically with the breakdown of these cells. Progressively greater inhibition of the rate of increase in packed cell volume, DNA and protein synthesis was observed at all concentrations to 175 γ/ml, from time of initial contact. Although limited inhibition was noted at the 125 and 150 γ/ml, losses in DNA, protein and PCV were not observed during the 24-hour test period. Cells exposed to final concentrations of 100 γ/ml are suppressed to only a slight degree as compared to the rates of cell syntheses seen in the control cultures. Over-all, the results indicate a clear effect of ABS on cell growth that is concentration-dependent.

Since 100 γ/ml exhibited only a slight suppression, over the test period, it appeared reasonable to determine if ABS could exert

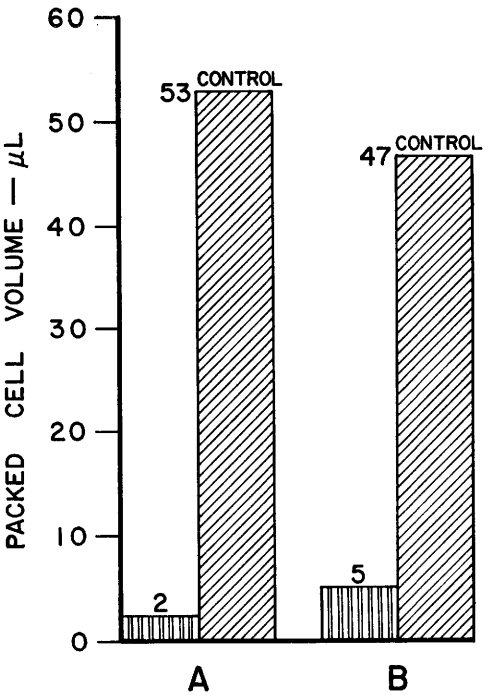

FIG. 2. μl of packed KB cells after extended contact with 100 γ/ml of ABS. (b) μl of packed KB cells remaining after daily contact with increasing increments of ABS.

cytopathological growth and inhibitory effects as a consequence of its accumulation by the cells over an extended contact period. To determine if contact with a relatively non-toxic dose could elicit the toxic response after prolonged contact, media containing 100 γ/ml final concentration were added to growing cell monolayers; these media were changed daily. Fig. 2a shows that control cultures, ABS-free, continually increase in packed cell mass during the entire test period. At the end of 8 days the cultures contained 53 μl of packed KB cells. On the other hand, in cultures exposed for the increased test period, to 100 γ/ml, ABS were inhibited. At the sixth day, morphological changes were clearly apparent in areas of the cell-sheet. By the seventh day, the effect had progressed to the entire sheet, and 2 μl of packed cells were obtained from the glass surface on the eighth day. This suggested that a relatively non-toxic level of ABS could exhibit a toxic reaction when in contact with growing cells for an extended period.

Cell growth is inhibited by ABS when it is fed to growing cell cultures in step-wise increments, starting with 20 γ/ml (Fig. 2). Degenerative cellular changes were observed by the fifth day, when the culture contained 120 γ/ml. This demonstrates that the toxic effects can be observed in 24 hours with 120 γ/ml if the culture is previously primed with ABS at lower levels, since 125 γ/ml as shown in Fig. 1 is relatively non-toxic in 24 hours.

Crude ABS is a commercial preparation which contains ancillary material to assist detergency and perform specific duties such as whitening and perfuming. These additives could reasonably enhance the toxicity of the surfactant. One hundred γ/ml final concentration of the crude ABS was no more suppressive over the short test period than the purified ABS.

Although morphological changes and physiological responses were seen with increased doses and time of contact with ABS and KB cells, it may not be inferred that these cells are equivalent to cells *in situ,* or that the mechanism of response is similar. Cells in culture can measure cytotoxicity and thus be a guide for screening chemicals, but, not being a whole animal, it is difficult to equate results to the intact animal, just as it is difficult to equate animal testing to human toxicity. Although the use of tissue culture for testing drugs and food additives is relatively new, Savchuck *et al*(3) and Smith *et al*(4) have reported the quantitative effect of arsenicals, antibiotics and fermentation beers on the growth of several types of mammalian cells in tissue culture.

These investigators used total protein determinations as the sole criterion of inhibitory effect. The study reported herein shows packed cell volume changes to be a sensitive indicator of cellular response. In fact, it appears to be more clear cut than either protein or DNA. Both DNA and protein determinations are involved chemical procedures, requiring numerous repetitions for significant data. On the other hand, P.C.V. offers a relatively straightforward method for assaying a toxic response.

Summary. An established KB cell-line in tissue culture was used as a means of obtain-

ing a more rapid indication of toxic responses than is presently available through classical toxicological animal feeding studies. The method of tissue culture and the application of growth analyses, *i.e.*, DNA, protein, and P.C.V., offer a quantitative means of detecting cytotoxicity before morphological changes become evident. Tissue culture offers a screening procedure capable of indicating substances requiring additional testing by more intensive methods, and a packed cell volume assay appears to offer a rapid direct procedure. ABS was found to be growth-suppressive and evocative of cytopathological changes, at several concentrations. In addition, these reactions were produced when relatively non-toxic levels were in contact with the growing cells for an extended period: 6 days in this series of experiments. Again, non-toxic levels showed similar toxic patterns when added in increasing increments, step-wise fashion, daily.

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Nerve Growth Factor Requirement for Development of Dissociated Embryonic Sensory and Sympathetic Ganglia in Culture. (29373)

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Nerve growth factor (NGF), a specific protein material extracted from mouse salivary glands, stimulates the growth of embryonic sensory and sympathetic nerve cells (1,2). Earlier, Nakai(3) experienced little success in attempting to grow enzymatically dissociated dorsal root ganglia in culture, but recently Levi-Montalcini and Angeletti(4) pointed out the requirement of NGF for survival and maintenance of dissociated embryonic sensory and sympathetic nerve cells in culture.

This paper concerns the finding that NGF under special culturing conditions enhanced the later development and organization of similar dissociated ganglia from single cell cultures into ganglia-like masses.

Materials and methods. Tissue culture. The entire lateral lines of dorsal root and sympathetic ganglia of 8-day-old chick embryos were dissected, cleaned, and teased apart with fine forceps. For dissociation of the ganglia, 0.15% Pancreatin (Wilzyme 600, Wilson Laboratories) was used. In early experiments the agitation was carried out at 37°C for 45 minutes (one fraction only). This resulted in complete dissociation of the tissue but yielded many supporting cells in culture. However, it was later found that agitation at 37°C using 3 $\times$ 20 minute fractions and plating only the third fraction after centrifugation produced cultures nearly completely free of supporting cells. Approximately 2.5 $\times$ 10^5 cells were plated on plastic petri dishes (Falcon disposable) some of which contained uncoated glass coverslips. A total of 4 ml of growth medium was used per plate and the incubation carried out in a gas exchange incubator in a 5% CO_2 atmosphere at 37°C.

Various media were tested to determine optimal growth effects on the cells (Eagle, 199, 1066 and tryptose phosphate with various concentrations of different sera). The medium of choice consisted of 0.5% lactalbumin hydrolysate (Difco) dissolved in Earle's salt solution fortified with vitamins, glutamin, 0.053 M glucose and antibiotics. The