The distribution of collagenous and non-collagenous protein in the cells and in the culture medium is shown in figure 2. Each point represents the average of paired samples. Endotoxin increased both collagenous and non-collagenous proteins both in the cell layer and in the culture medium. The increase was dose-dependent with a greater synthesis of collagen in the cell layer versus the culture medium, whereas the non-collagenous protein synthesis was greater in the culture medium versus the cell layer.

The effect of endotoxin on cell layer collagen extractability (figure 3) showed a slight but consistent increase in the saltsoluble fraction at the expense of the insoluble fraction; the acid-soluble fraction is relatively unchanged. The changes in the salt-soluble and insoluble fractions do not appear to be dose-related, at least at the concentrations used in these experiments.

In the endotoxin-treated cells, there is very little difference in the proline/hydroxyproline ratios in the acid-soluble and insoluble fractions, but the proline/hydroxyproline ratios of the salt-soluble fraction are definitely elevated signifying underhydroxylation. The macromolecules found in the medium are also underhydroxylated, but the degree of underhydroxylation in this fraction or in the salt-soluble fraction does not appear to be dose-related.

Discussion. These data show that endotoxin, in vitro, in the absence of the usual activation of an immune effector system found in vivo, can alter the synthetic capabilities and depress the proliferation of fibroblasts. This direct action of endotoxin may be due to its affinity for membranes. As a lipopolysaccharide, endotoxin is capable of interacting with phospholipids found in cell membranes[8] and thus, has a profound effect upon the physiological responses of the cell. Modified cell membranes may disrupt or alter membrane-bound enzymes, or contribute to a host of other metabolic alterations brought about by changes in membrane transport.

The hypermetabolism of fibroblasts induced by endotoxin in this study resembles the changes seen by Buckingham and Castor[9] who investigated the effects of extracts of Gram-negative bacteria on fibroblast cultures. They found a marked increase in hyaluronic acid production, glucose utilization and lactate output. Their studies demonstrated that endotoxin was bound to fibroblasts, but they had no conclusive evidence of how the metabolic changes occured. Whether membrane changes were solely responsible or whether the production of mediators resulted in hypermetabolism is not clear.

This study shows that endotoxin has a direct effect upon the metabolism and proliferation of a homogenous fibroblast population apart from the controlling and modifying influences of the host. The data presented may contribute to the understanding of the chronicity of periodontal disease. If cementum-bound endotoxin is allowed to remain in contact with the supporting tissues of teeth, the depression of cellular proliferation and the alterations in tissue metabolism make the prognosis of early eradication of the disease unlikely, and the development of the disease into chronic phases inevitable.

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The chromosome banding patterns of the aardvark *Orycteropus afer* (Tubulidentata, Orycteropidae)[1]

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Summary. C- and G-banding patterns of *Orycteropus afer* are described on the basis of fibroblast cultures obtained from a female individual.

Benirschke et al.[2], based on their conventional preparations, reported that the diploid chromosome number of the aardvark, *Orycteropus afer*, is 20. Its karyotype consists of 2 pairs of large subtelocentric plus 7 pairs of medium to small metacentric and submetacentric autosomes, and the sex pair. The X is a small metacentric and the Y is the smallest biarmed chromosome. We report here the C-band and G-band patterns of this species.

Skin biopsies of 2 sister aardvarks were obtained from Point Defiance Zoo in Tacoma, Washington. 1 sample was contaminated but the other gave rise to fibroblast cultures from which cytological preparations were made. C- and G-band procedures followed those described earlier[3].

From more than 100 metaphase plates in conventionally Giemsa-stained slides, we confirm the diploid number and the karyotype described by Benirschke et al. All chromosomes possess centromeric C-bands, but pairs 4 and 7 have an additional terminal C-band in the long arm, and pair 6 has an additional C-band in the short arm (figure 1). These additional C-bands are not obvious in the photograph, but they were consistently observed in our preparations. The amount and distribution of the C-band material in the aardvark karyotype is not unusual.

In the absence of male cells, the identification of the X chromosome in our karyotype follows that of Benirschke et al., hence the ? in figure 2, a G-band karyotype. Every chromosome pair can be unequivocally identified by G-banding.

Armadillos, anteaters, pangolins and the aardvark were originally classified into the single order Edentata. Subsequently, the pangolin was placed into a separate order Pholidota, and the aardvark, into another order Tubulidentata. The diploid number of the armadillos ranges from 58 to 64[4,5], and that of the anteaters ranges from 54 to 60[6]. A

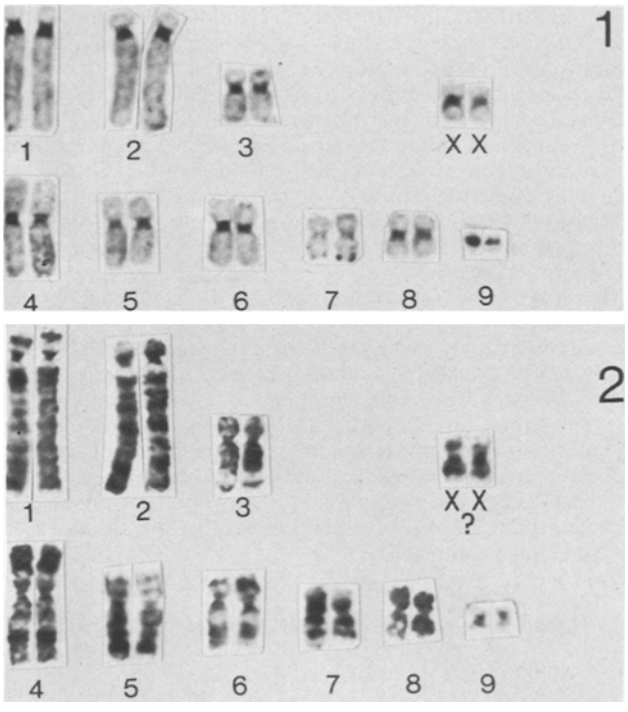

Fig. 1. A C-banded karyotype of a female metaphase of aardvark. Note the presence of centromeric heterochromatin in every pair of chromosomes. Pairs Nos 4 and 7 have terminally located C-bands in the long arms whereas pair No. 6 has terminally located C-bands in the short arms. Pair No. 9 has polymorphic C-banded segments.

Fig. 2. A G-banded karyotype prepared from a female metaphase plate. Each pair of chromosomes has its own distinctive banding pattern. The identification of the 2 X chromosomes is not certain.

single species of the pangolin, *Manis pentadactyla*, has a diploid number of 36[7,8]. The diploid number of 20 for the aardvark is, therefore, the lowest among these animals. Undoubtedly numerous rearrangements must have taken place in the karyotypic evolution among these animals.

Using the microspectrophotometric measurement technique to estimate the DNA content per cell, Benirschke et al.[2], showed that the aardvark nuclei had 1.673 more DNA than human nuclei. In eutherian mammals, the DNA content per cell is more or less the same. In cases where a species may have a higher DNA content than its related species (e.g., *Peromyscus eremicus* vs *P. crinitus*), the difference can always be attributed to the amount of C-band[9]. Since the aardvark chromosomes do not display an unusual amount of constitutive heterochromatin, the unusually high DNA content in Benirschke's material requires confirmation.

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Hemolymph volume determination in the tomato fruitworm, *Heliothis zea*

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Summary. The hemolymph volume of *Heliothis zea* larvae was determined by the amaranth dye method and found to vary from 28 to 288 µl. It averaged 30.6% of b.wt, a value comparable to that obtained with C^{14} inulin. Amaranth became evenly distributed in the hemocoele in 3–5 min.

In the course of physiological and toxicological studies on insects, it is often useful to know the volume of the hemolymph. Reported here is a modification of the dye dilution method[1,2] applied to immature tomato fruitworms (TFW), *Heliothis zea* (Boddie). Workers in this area consider this to be a reliable technique[3,4].

Methods. TFW larvae were raised singly in 60-mm plastic petri dishes on an artificial diet (Bio-Serv®, Frenchtown, N.J. 08825). Larvae were selected from mid-stadia of the last 3 instars for experimentation. Individuals were injected with 10 µl of an amaranth dye solution containing 20 mg dye/ml aqueous NaCl. Injections were made under a 60× binocular microscope using a microhypodermic needle made from a 50-µl disposable glass pipet drawn to a tip diameter of ca. 250 µm. The needle was connected to a 100-µl calibrated glass syringe via segments of polyethylene tubing of decreasing diameters. Larvae were restrained during injection by being partially wrapped in tissue paper and held between the thumb and forefinger. The injection site was the dorsal vessel between the 11th and 12th body segments. This location was chosen because it avoided loss of dye and hemolymph (in case of loss, the larvae was discarded) and it facilitated the rapid dispersal of the dye. During dye circulation, the animal was weighed. The dye circulation period could be decreased by gently massaging the specimen. Within 5 min of injection, a 10-µl aliquot of dyed hemolymph was collected from a severed proleg using a disposable micropipet. It was then mixed in 1.0 ml of a 0.5% sodium dodecyl sulfate (SDS) solution in distilled water, which lyzed the cells and cleared the sample. Centrifugation was not found to be necessary for consistent results. Standards containing from 0.5 to 40.0 µg dye and 10 µl of untreated hemolymph per ml of SDS solution behaved in accordance with Beer's Law, when read at 515 nm. Samples and standards were read on an American Optical Spec 20® spectrophotometer.

Results. Amaranth dye became evenly distributed in about 3 min after injection. Hemolymph volume was calculated