line. That the urinary output of hydroxyproline is not always elevated in dermatomyositis is evident from the normal excretion of patient No. 20 who from a clinical standpoint had active dermatomyositis at the time of observation. It should be noted that these patients were receiving cortical steroids.

It cannot be assumed that the lower excretion of hydroxyproline in pseudohypertrophic muscular dystrophy is due to accumulation of collagen in the muscles, although the results do not rule out this possibility. A relationship between rate of growth of the individual and excretion of hydroxyproline has been postulated(8). Whether the mechanisms operating during growth are altered in muscular dystrophy is under investigation.

Summary. Endogenous collagen metabolism was studied in 9 patients with pseudohypertrophic muscular dystrophy between 6 and 18 years of age, and in 11 patients with other diseases of the muscles, by measuring urinary total hydroxyproline excretion. The results obtained in pseudohypertrophic muscular dystrophy indicate that excretion of total hydroxyproline is significantly less at all ages than in controls of the same age. Two girls, 11 and 17, one with facio-scapulohumeral muscular dystrophy, and the other with the limb-girdle type of the disease, apparently have a lower excretion of hydroxyproline than controls of the same age. The other patients, all adults, have a normal excretion, with the exception of one patient with dermatomyositis. The significance of these results is discussed.

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1. Weinstock, I. M., Epstein, S., Milhorat, A. T., PROC. SOC. EXP. BIOL. AND MED., 1958, v99, 272.

2. Ziff, M., Kibrick, A., Dressner, E., Gribetz, H. J., *J. Clin. Invest.*, 1956, v35, 579.

3. Kibrick, A. C., Hashiro, C., Schutz, R. S., Milhorat, A. T., *Fed. Proc.*, 1963, v22, 648.

4. Milhorat, A. T., Wolff, H. G., *Arch. Neurol. Psychiat.*, 1937, v38, 992.

5. Kibrick, A. C., Milhorat, A. T., *Intern. Congr. Clin. Chem.*, Detroit, August 1963.

6. Prockop, D. J., Udenfriend, S., *Anal. Biochem.*, 1960, v1, 228.

7. Jones, C. R., Bergman, M. W., Kittner, P. J., Pigman, W. W., PROC. SOC. EXP. BIOL. AND MED., in press.

8. Jasin, H. E., Fink, C. W., Wise, W., Ziff, M., *J. Clin. Invest.*, 1962, v41, 1928.

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Effect of Radioisotope Disintegration on Cell Culture Cell Viability.* (29000)

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Stent and Fuerst(1) have reviewed the applications of the decay of biologically incorporated radioisotopes to genetic and physiological studies in phages and bacteria. By controlled incorporation of a radioisotope into a particular biochemical compound and by using suitable experimental manipulations one may obtain transmutation of one element into another *in situ* and determine the biological consequences of transmutation under varying physiological conditions. If applicable to mammalian cells in culture, studies similar to those reviewed by Stent and Fuerst(1) could be made with animal cells.

The functioning of the blueprint, DNA, for phage synthesis has been examined in detail by Stent and his co-workers(2,3) using ^{32}P decay. They found that the information was transferred to a structure not inactivated by ^{32}P decay after 9 minutes under growing conditions and that protein synthesis was required for this transfer.

These studies have rarely been extended

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to higher organisms except for the observations that growth in a solution containing a high level of ^{32}P was abnormal and that the ^{32}P disintegrations were mutagenic in *Drosophilia* and *Habrobracon* (4,5,6).

A priori mammalian cells would presumably be more sensitive to decay of ^{32}P than microbial cells since the β particle emitted has a longer track inside the mammalian cells, thus superimposing ionizing effects onto that of nuclear recoil and transmutation. The wider function of phosphorus-containing compounds in mammalian cells might be expected either to increase or decrease their sensitivity to this radiation depending upon the essentiality and number of replicas of the incorporating material.

Carrier free ^{32}P as phosphate was obtained from the Oak Ridge National Laboratory and was sterilized by autoclaving. Phosphate-free medium 199 was obtained from Microbiological Associates, Bethesda, Md., and for the purposes of this experiment it was supplemented with 1 mμmole per ml of $PO_4^{\equiv}$. Varying concentrations of the radioactive phosphate solution were added to cultures of KB cells (obtained from Dr. Vernon Scott, Univ. of Oklahoma Med. School) growing in milk dilution bottles to obtain a range of specific activities between 0 and 50 mC per mg P. The cells were grown for 48 hours in the radioactive medium, then washed once with a balanced salts solution. Treatment with trypsin-versene was used to obtain single cells which were harvested by centrifugation. The cells were enumerated using a Coulter Cell Counter and appropriate aliquots were plated in Falcon petri dishes containing medium 199 with 10% calf serum; after 2 weeks' growth they were stained with haemotoxylin for colony enumeration. All results were obtained by plating efficiency experiments as described above. Other aliquots of the cells grown in the presence of ^{32}P were suspended in medium 199 containing 10% calf serum and 10% glycerol and stored until required in a liquid nitrogen refrigerator. After 4, 9, 20, 30 and 42 days storage the samples were thawed and appropriate aliquots plated for determination of efficiency of killing.

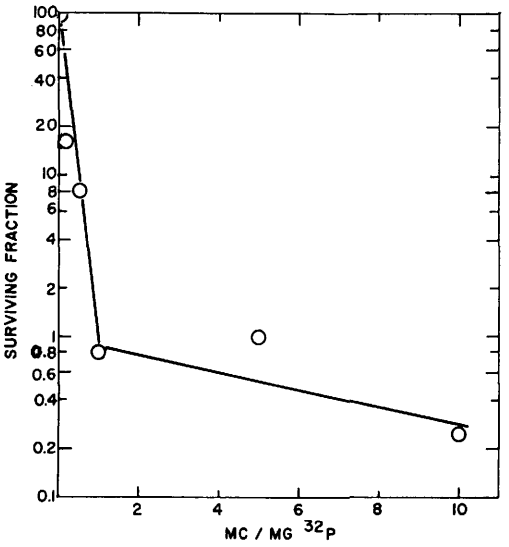

FIG. 1. Effect of ^{32}P on relative plating efficiency of KB cells. KB cells were grown in ^{32}P containing medium 199 with 10% calf serum for 48 hr as described in text. Suitable aliquots were counted using a Coulter Cell Counter and plating for determination of viability.

Fig. 1 shows the effect of ^{32}P concentration on the viability of cells after 2 days' growth in the presence of ^{32}P. This plot shows the typical radiation sensitivity form. The diphasic nature of the curve suggests that there are 2 kinds of cells in the culture as far as sensitivity to ^{32}P decay. This might be due to variation in ploidy or in genotypes. Variation in cell and colonial size and morphology as well as drug resistance in normal KB cell populations suggests that several genotypes are present (unpublished observations). That the killing measured in these experiments occurs because of incorporation of radiophosphorus into cellular constituents was demonstrated by the fact that storage of non-growing cells in medium containing 0.3 and 3.0 mC/mg did not result in a significant decrease in viability. With activities greater than 1 mC/mg the efficiency of killing did not increase as expected during this 2-day exposure.

Table I summarizes the efficiencies of killing by ^{32}P decay for various organisms. In øX-174 every decay results in killing because the DNA of this phage is single stranded and is broken by each decay. In coli phage T2

TABLE I. Efficiencies of Killing by ^{32}P Decay.

Organism	Investigator	α (efficiency)
øX-174	Tessman(8)	1.1
Phage T2	Stent and Fuerst(9)	.12
E. coli	Fuerst and Stent(10)	.02
D98/AG	Ragni and Szybalski(7)	.00023
KB	Leach	.0004*

Calculated from the equation $\log_{10}s = -1.48 \times 10^{-6}\ \alpha\ A_0N(1-e^{-\alpha t})$ in which s is number of survivors, A_0 is specific activity of the medium in mC/mg P, N is number of phosphorus atoms per cell in DNA, and t is number of days. A value of 2×10^{10} DNA-P atoms was used in this study.
* Calculated from 0.1 and 0.5 mC/mgP levels.

about every tenth disintegration is lethal and Stent and Fuerst have postulated that to be lethal a decay must occur opposite a naturally occurring break in the double stranded DNA for chain breakage. *E. coli* is more resistant, possibly because it has several nuclei under common growth conditions which could undergo intracellular recombination to maintain viability. A study of the effect of bromodeoxyuridine substitution on mammalian cell radiosensitivity by Ragni and Szybalski(7) reveals that the Detroit 98 strain of cells is also radioresistant. They found only one level of resistance—not a biphasic curve. The possibilities resulting in greater resistance are that only a small portion of the genome must function for a mammalian cell to plate (give rise to a colony) under the tissue culture conditions imposed and that the cells are polyploid. The polyploid nature of the cells makes possible intracellular recombinations to yield the same result as multiplicity reactivations in phages. Similar postulates were made by Ragni and Szybalski(7). As would be expected from the previous data the efficiency of killing of cells labeled with ^{32}P activities greater than 1 mC/mg was lower than 4×10^{-4}. However, their viability decreased with time (decay of ^{32}P) suggesting that the more resistant cells also contained ^{32}P.

The lack of sensitivity of mammalian cells cultured *in vitro* to ^{32}P decay when incorporated into DNA makes impractical the application of this tool in the elegant way of Stent and co-workers. The existence of cells with 2 levels of sensitivity to ^{32}P disintegration in mammalian cell cultures is described.

1. Stent, G. S., Fuerst, C. R., *Advanc. Biol. Med. Phys.*, 1960, v7, 1.
2. Uchida, H., Stent, G. S., *J. Mol. Biol.*, 1960, v2, 251.
3. ———, *ibid.*, 1960, v2, 262.
4. Grosch, D. S., Sullivan, R. L., *Biol. Bull.*, 1953, v105, 296.
5. King, R. C., *J. Exp. Zool.*, 1954, v126, 323.
6. Oftedal, P., Mossige, J., *Nature*, 1957, v179, 104.
7. Ragni, G., Szybalski, W., *J. Mol. Biol.*, 1962, v4, 338.
8. Tessman, I., *Virology*, 1959, v7, 263.
9. Stent, G. S., Fuerst, C. R., *J. Gen. Physiol.*, 1955, v38, 1441.
10. Fuerst, C. R., Stent, G. S., *ibid.*, 1956, v40, 73.

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Passive Transfer of Human Allergies to Prosimians: Skin-Reactions in the Lemuroid, *Nycticebus coucang* (Slow Loris). (29001)

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Layton and coworkers have shown(4,5) that human atopic hypersensitivities of asthma and hay fever can be passively transferred by serum from allergic human beings to nonhuman primates of suborder *Anthropoidea*. Most species of New World monkeys and Old World monkeys, apes, and men exhibit essentially the same capacities to bind atopic reagins of human sera in skin and to exhibit reactions in the passively sensitized sites when challenged with the appropriate atopens (allergens).

Anthropologists generally believe that the

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