p,p′ DDD from *p,p′* DDT. These results suggested a possible reason for the high concentration of *p,p′* DDD in fish liver oils. The ability to dechlorinate *p,p′* DDT may not be limited to yeast but may also reside in higher animal forms. Although the conversion of *p,p′* DDT to such metabolites as DDE (*3*) and DDA (*4*) has been reported for species from the fly to man, there has been no evidence thus far that *p,p′* DDT can be dechlorinated to *p,p′* DDD in forms higher on the evolutionary scale than yeast. In this report we present gas chromatographic data that such a conversion occurs in at least one mammalian species, the rat.

Eighteen mature male and female Sprague-Dawley rats, each weighing about 210 to 320 g, were divided into three groups in which the members of each were matched in weight and sex. One group served as controls, and the animals were maintained on a standard diet of ground Purina laboratory chow. The two experimental groups were maintained on the same diet, but *p,p′* DDT or *p,p′* DDE (50 parts per million each) was added. Each experimental rat consumed approximately 0.75 mg of *p,p′* DDT or *p,p′* DDE daily.

After 12 weeks on these diets the animals were killed, and the livers, kidneys, and abdominal fat were removed for analysis and weighed. The tissues were extracted with ethyl ether and each extract was made up to 50 ml. A portion was evaporated, and then the residue was taken up in petroleum ether distilled from an all-glass system and treated with acetonitrile (*5*) to remove fat. Although gas chromatography of the acetonitrile-treated petroleum ether extracts clearly distinguished between *p,p′* DDT and *p,p′* DDE, the peak for *p,p′* DDD, if present, exactly coincided with that for *p,p′* DDT. In order to distinguish between *p,p′* DDT and *p,p′* DDD the method of Klein and Watts (*6*) was employed. This consisted in preparing the corresponding olefins by hydrolysis of the defatted tissue extracts with alcoholic sodium hydroxide.

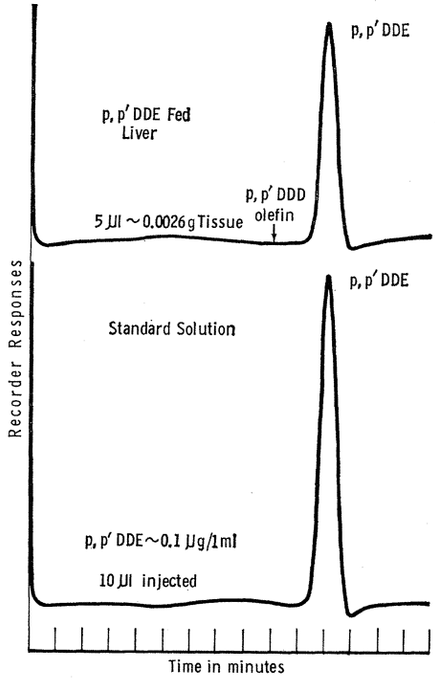

Fig. 2. Gas chromatogram of a defatted liver extract from a rat fed *p,p′* DDE.

The gas chromatographic analyses were carried out in a Jarrell-Ash model 700 gas chromatograph with a 90-cm U-shaped glass column which was mildly polar. The column consisted of a 2.5 percent coating of a mixture of silicone fluid 96 (General Electric) and 2,2-diethyl-1,3-propanediol-isophthalate polyester (1:1 by weight) on Celite. The column temperature was set at 175°C, the electron capture detector at 200°C, the splitter and injector units at 190°C, and an 18-volt potential was applied to the cell. The system was able to detect readily 5×10^{-4} µg of the olefin of *p,p′* DDT, 2.5×10^{-4} µg of the olefin of *p,p′* DDD, and 5×10^{-4} µg of the olefin of *o,p* DDT.

If the results for tissues of rats fed *p,p′* DDT (Fig. 1) are compared with the result for liver tissue from a control rat, it is evident that considerably more *p,p′* DDD is present in the liver of rats fed *p,p′* DDT. It should be noted, however, that under the conditions of these experiments no *p,p′* DDD was observed in either kidney or fat. Traces of *p,p′* DDD were also observed in the liver of the control animal as well as *p,p′* DDT and *o,p′* DDT. This is attributed to the presence of mixed DDT residues in the commercial rat chow. *p,p′* DDD was found in the livers of all experimental and control rats, and there was no demonstrable difference between the amounts found in males and females. Figure 2 shows that the liver of a rat fed *p,p′* DDE does not contain *p,p′* DDD. No *p,p′* DDD was found in the kidneys or fat of rats fed *p,p′* DDE.

It seemed of interest to compare the relative amounts of *p,p′* DDD olefin and total *p,p′* DDT olefins present in livers of rats fed *p,p′* DDT. In six rats, where a graphic analysis was made, the ratio of *p,p′* DDD to total *p,p′* DDT olefins is approximately 1:1. However, when account is taken of the *p,p′* DDE [*p,p′* DDT olefin] which was already in the liver as a result of metabolic processes, then a ratio of the *p,p′* DDD to unconverted *p,p′* DDT stored in the liver is approximately 2:1.

Our results thus show that *p,p′* DDT is converted to *p,p′* DDD in the rat liver and that this conversion does not require the intermediate formation of *p,p′* DDE.

P. R. Datta
E. P. Laug
A. K. Klein

Food and Drug Administration, U.S. Department of Health, Education, and Welfare, Washington 25, D.C.

References and Notes

1. Food and Drug Administration, unpublished reports.
2. B. J. Kallman and A. K. Andrews, *Science* **141**, 1050 (1963).
3. A. W. Lindquist, A. R. Roth, W. W. Yates, R. A. Hoffman, J. S. Butts, *J. Econ. Entomol.* **44**, 167 (1951); A. M. Mattson, J. T. Spillane, C. Baker, G. W. Pearce, *Anal. Chem.* **25**, 1065 (1953).
4. J. D. Judah, *Brit. J. Pharmacol.* **4**, 120 (1949); W. C. White and T. R. Sweeney, *Public Health Rept. U.S.* **60**, 66 (1945).
5. P. A. Mills, J. H. Onley, R. A. Gaither, *J. Assoc. Offic. Agr. Chemists* **46**, 186 (1963).
6. A. K. Klein and J. O. Watts, *ibid.* **47**, 331 (1964).

7 July 1964

Redifferentiation of Connective Tissue Cells in Serial Culture

Abstract. *When cell cultures are initiated from aortic connective tissue of rats and maintained as monolayers on glass, the differentiated property of collagen production disappears. However, these same cells, when placed in diffusion chambers in the peritoneal cavities of other rats, produce collagen, as indicated both morphologically and by the accumulation of hydroxyproline within the chambers.*

Some of the early workers in tissue culture, notably Champy (*1*), regarded the loss of identifiable morphologic and biochemical characteristics of differentiation, which occurs in tissue culture, as a process of dedifferentiation —from which state cells could again return. With the more recent common use of cell culture techniques this concept of dedifferentiation and redifferentiation has been questioned (*2*). Although specialized functions such as synthesis of collagen (*3*) or acid mucopolysaccharides (*4*) will, in rare instances, persist when cells are grown

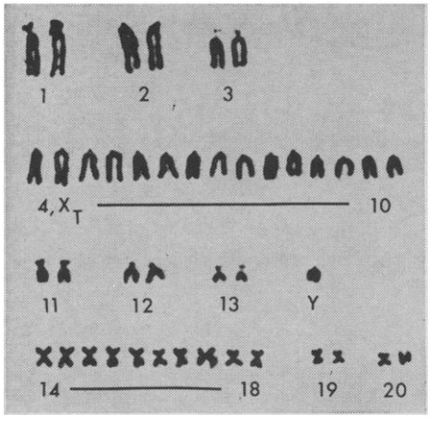

Fig. 1. Karyotype of diploid cultured cell from a male rat showing 42 chromosomes, X_T and small positively heteropyknotic Y. Pairs 3 and 13 are satellited.

as monolayers on glass or in suspension cultures, they more commonly disappear. When cells in serial culture lose such specialized functions they have not been observed to regain them. Evidence presented in this report shows that connective tissue cells may regain the differentiated function of collagen synthesis that has been lost during many serial passages as monolayers on glass.

We grew the cells used in these experiments from aorta of the rat by explantation into plasma clots, transferred them to prescription bottles, and maintained them for several months in serial culture as monolayers on glass. The nutrient medium was F-10 (*5*), supplemented with 10 percent fetal calf serum and 5 percent fetal human serum.

The culture medium was changed two times a week, and trypsinization and subdivision were carried out when growth became confluent. Cell doubling time was about 40 hours. The cells retained the normal diploid complement of 42 chromosomes (Fig. 1) for about 4 months, as long as they were observed (*6*). Analysis of the medium and cells during this time revealed no accumulation of collagen, as measured by the content of hydroxyproline, even in cultures allowed to remain confluent for several days.

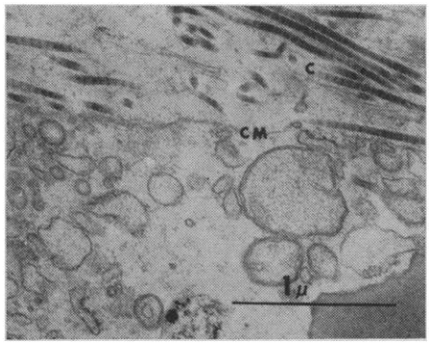

Fig. 2. Electron micrograph showing collagen bundles (*C*) near the cell membrane (*CM*).

Cells maintained in this way through 15 or more transfers were placed in diffusion chambers (*7*). The chambers were constructed by cementing a Millipore filter (pore diameter 0.22 μ) on one side of a Plexiglas disk 2.5 cm in diameter and 0.3 cm deep. The chamber was sterilized with ultraviolet light and 0.25 ml of growth medium that contained 5×10^5 cells was placed in the chamber. It was then closed by cementing another filter membrane to the other side. Two chambers were inserted into the peritoneal cavity of each rat. After 3 weeks a thin fibrous capsule surrounded the chamber. Within the chamber a layer of tissue six to ten cells thick was found adjacent to the filter membrane. The elongated cells forming the tissue within the chamber were separated from one another by an abundant extracellular material, part of which showed an affinity for the aniline blue of the Mallory trichrome stain and for light green of the Gomori trichrome stain. Both stains are used to show collagen. Electron micrographs reveal numerous fibers with a major periodicity of 640 Å, which is a characteristic of collagen (Fig. 2). Chambers that did not contain cells originally had only fluid that resembled plasma.

The thin fibrous capsules were removed from outside the chambers, and filter membranes and layers of tissue inside the diffusion chambers were hydrolyzed and analyzed for hydroxyproline, a component of collagen (Table 1) (*8*). The small amount of hydroxyproline present on the filter membranes from blank chambers probably was a result of contamination from the fibrous capsules.

Our results indicate quite clearly that at least one differentiated function, the production of collagen, can be regained by cultured cells following its disappearance. The genetic capacity of the cells to produce collagen is therefore not irreversibly lost during the period of growth as monolayers on glass. Because the ability to produce collagen is retained through 15 or more transfers in culture, by which time homogeneity of the cell population might be expected, it is probable that this capacity is a property of all the cultured cells rather than of only some.

Table 1. Hydroxyproline in diffusion chambers.

Empty chamber (μg hydroxyproline per chamber)	5×10^5 cells introduced (μg hydroxyproline per chamber)
18.6	176.0
18.0	135.0
6.4	129.0
34.0	124.0
11.6	138.0
16.0	156.0

ROBERT E. PRIEST
JEAN H. PRIEST
Department of Pathology and
Department of Pediatrics, University of Colorado Medical Center, Denver

References and Notes

1. C. Champy, *Bibliog. Anat.* **23**, 184 (1913).
2. C. Grobstein, in *The Cell*, J. Brachet and A. Mirsky, Eds. (Academic Press, New York, 1959), vol. 1, pp. 479–480.
3. B. Goldberg, H. Green, G. Todaro, *Exptl. Cell. Res.* **31**, 444 (1963).
4. C. Morris, *Ann. N.Y. Acad. Sci.* **86**, 878 (1960); H. Green and D. Hamerman, *Nature* **201**, 710 (1964).
5. R. Ham, *Exptl. Cell. Res.* **29**, 515 (1963).
6. D. Hungerford and P. Nowell, *J. Morphol.* **113**, 275 (1963).
7. R. Curran and E. Rowsell, *J. Pathol. Bacteriol.* **76**, 561 (1958).
8. D. Prockop and S. Udenfriend, *Anal. Biochem.* **1**, 228 (1960).
9. This work was supported in part by a grant from the U.S. Public Health Service.

29 June 1964

C-Phycocyanin: Minimum Molecular Weight

Abstract. *Sedimentation and immunodiffusion experiments indicate that the molecular weight for the minimum molecular unit of C-phycocyanin is 30,000. This result agrees with an analysis of available data on amino acid content for C-phycocyanins from several different algae.*

The molecular weight and other properties of phycocyanin, a biliprotein from blue-green and red algae have been studied (*1*). Svedberg *et al.* (*2*, *3*) investigated the molecular weights of C-phycocyanin and other phycocyanins as a function of *p*H by sedimentation velocity and sedimentation equilibrium studies. Svedberg and Katsurai (*2*) estimated the molecular weight for C-phycocyanin as 208,000 and for the