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Failure of Deoxyribonucleic Acid to Effect Somatic Transformation in the Rat.* (24476)

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It is well established that isolated deoxyribonucleic acid (DNA) can produce heritable changes in several species of bacteria. This was first demonstrated by Avery *et al.*(1), who found that DNA extracted from a smooth (encapsulated) strain of *Pneumococcus* would transform rough (unencapsulated) cells into smooth cells. The capsule of the newly modified cells was serologically the same as that of the strain from which the DNA had been derived, and the conversion could be brought about in a rough pneumococcal strain which had not been observed to mutate spontaneously. In addition, the transformed cells propagated indefinitely as a smooth strain without further treatment with DNA. The pneumococcal DNA preparation had performed 2 functions usually attributed to genes: it had induced a specific heritable property, and had initiated its own reduplication. Subsequently other investigators demonstrated ability of isolated DNA to effect transformations in other species of bacteria, *viz. Hemophilus influenzae, Meningococcus, Escherichia coli,* and the crown-gall organism *Agrobacterium*(2). Benoit *et al.*(3,4) recently have reported that somatic changes are produced in one strain of ducks by injection of DNA derived from another strain, and that the changes were transmitted to progeny of the treated birds. The present paper describes an attempt to produce a similar transformation in mammals. This work was stimulated by the prospect that a successful result might have application to the treatment of genetically determined diseases in man.

Material and methods. Preparation of DNA. DNA was isolated from thymuses and spleens of rats by a modication of the method used by Benoit *et al.* (personal communication) to prepare DNA from duck erythrocytes and testicular tissue.† Adult rats were killed, and the spleen and thymus were immediately removed and frozen over dry ice. All further steps in isolation of DNA were carried out in a cold room at 0°C or in a refrigerated centrifuge. The following solutions were used in the preparation of DNA: I. Versene-fluoride: 18.7 g disodium versenate and 1.85 g sodium fluoride in 1000 ml water, adjusted to pH 7.0 ± 0.1. II. Fluoride-chloride: 58.0 g sodium chloride, 1.85 g sodium fluoride, and 8.0 g disodium versenate in 1000 ml water, adjusted to pH 7.0 ± 0.2. III. Versene: 3.75 g disodium versenate in 1000 ml water, adjusted to pH 7.0 ± 0.2. IV. Phosphate buffer, 2.5 M, pH 8.4. V. Sodium chloride solution, 0.85%. 100 g of frozen rat spleen and thymus was minced through a meat grinder and homogenized with 300 ml versene-fluoride solution in a Waring Blender for 5 minutes. The suspension was then centrifuged at 2500 rpm for 30 minutes, the supernatant liquid was discarded, and the sedimented nuclear fragments were collected. Homogenization and centrifugation were repeated as above 5 times. The nuclear fragments then were suspended in 400 ml fluoride-chloride solution and stirred overnight.

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† This modification was devised by Dr. Matthew Meselson, Calif. Inst. of Tech.

The suspension was centrifuged for 30 minutes at 30,000 g. The supernatant was separated and added to 20 ml 2.5 M phosphate buffer, and the mixture saturated with sodium chloride. The sediment from centrifugation was resuspended in 100 ml fluoride-chloride, stirred overnight, then centrifuged for 30 minutes at 30.000 g. To the supernatant, 5 ml 2.5 M phosphate buffer was added, and the solution was saturated with sodium chloride. The 2 salt-saturated solutions were combined, stirred for 3 days, then centrifuged at 80.000 g for 1 hour. The sediment was discarded and the supernatant filtered if not clear. The filtrate then was poured into 2 volumes of 95% ethanol with stirring and the white fibrous clot was collected on a wooden paddle. Excess alcohol was pressed out of the clot, which was redissolved in 100 ml versene solution by stirring overnight or longer. The resulting solution was centrifuged for 30 minutes at 30.000 g, the sediment was discarded, and the alcoholic precipitation was repeated. The final clot of DNA was dissolved with stirring in a minimal volume of 0.85% sodium chloride solution, and the resultant solution of DNA was dialysed against 0.85% sodium chloride solution to remove any traces of versene or fluoride. Approximate concentration of DNA in the final preparation was determined spectrophotometrically at 260 m, and the DNA solution was stored in the refrigerator until used. *Animals.* Three strains of rats were used: Addis-Slonaker, in which the animals always have pink eyes and white hair; Long-Evans, a hooded strain in which animals have black eyes, black heads and other patches of black hair on the coat; and the CNH mutant strain of hooded rat,‡ in which homozygous recessive individuals have a constitutional nonhemolytic hyperbilirubinemia. Homozygous recessive animals of the CNH strain become jaundiced within a few hours of birth, and the serum bilirubin level is always elevated. They are less hardy than their non-jaundiced (*i.e.* heterozygous or homozygous normal) litter mates, grow more slowly, and either fail to breed or produce small litters. Many of them are ataxic, possibly due to kernicterus. The hyperbilirubinemia of these rats has been shown to be due to a congenital absence in the liver of an enzyme, glucuronyl transferase, which is required for normal conversion of relatively insoluble bilirubin into soluble bilirubin glucuronide, which is excreted into the intestinal tract by way of the bile(5). Either subcutaneous or intraperitoneal injections of DNA were given to rats daily, starting on the first day of life. The solutions were highly viscous and were given in as high a concentration as could be injected through a 26 gauge hypodermic needle. The volume of solution injected was increased at intervals as the animals grew, and generally was the maximum which the animals seemed able to tolerate.

Results. Attempts to produce pigmentary changes in white rats. Nine newborn Addis-Slonaker rats, all litter mates, were selected for subcutaneous injection of DNA. Four rats were each given 17 injections of DNA derived from Long-Evans rats during the first 19 days of life, each rat receiving a total of 78 mg DNA. Three litter mates were each given 17 injections of DNA derived from Addis-Slonaker rats (their own strain) during the first 19 days of life, each rat receiving a total of 72 mg DNA. The remaining 2 litter mates served as uninjected controls. All developed normally, and the injected rats showed no adverse effect produced by DNA. None of the injected rats showed any change in normal white hair color or pink eyes.

Ten newborn Addis-Slonaker rats were used for intraperitoneal injection of DNA. Five were given Long-Evans DNA; 2 of these died on the 12th and 13th days, presumably as a result of the intraperitoneal injections. The 3 survivors each received a total of 18 injections of DNA in the first 22 days of life, totalling 57 mg/animal. Five litter mates served as uninjected controls. The 3 injected rats, as well as the controls, reached adult life, and none showed any pigmentary change.

Two rats which had been injected subcutaneously with Long-Evans DNA were subsequently bred with 2 rats which had been injected intraperitoneally with Long-Evans

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DNA. Both matings resulted in normal litters, and rats of the F_1 generations showed no change from normal pigmentation of the Addis-Slonaker rat.

Attempts to relieve hyperbilirubinemia with injections of "normal" DNA. Four jaundiced newborn litter mates of the CNH strain were given 16 subcutaneous injections of DNA derived from Addis-Slonaker rats, in which hyperbilirubinemia due to absence of glucuronyl transferase is unknown. Each rat received a total of 45 mg DNA during the first 18 days of life. These rats grew at a rate comparable to that shown by other jaundiced rats in the colony, and 2 developed obvious ataxia. No decrease in degree of jaundice was noted. Serum bilirubin levels were determined on these rats at the ages of 5, 10 and 15 weeks, by the method of Malloy and Evelyn(6) as modified by Kaplan and del Carmen(7). Serum bilirubin levels in each determination varied between 5 and 10 mg/100 ml, as did those of all tested uninjected jaundiced rats in the colony.

Two attempts were made to inject Addis-Slonaker DNA intraperitoneally into jaundiced newborn CNH rats. These rats tolerated intraperitoneal injections poorly, and on both attempts no rat survived long enough for serum bilirubin determinations to be done. Two animals survived a total dose of 47 mg DNA each in 16 daily injections, and 3 survived a total dose of 16 mg each given in 10 injections. These rats remained markedly jaundiced until they died at between 3 and 5 weeks of age.

Other attempts were made to test the possibility of influencing the embryo by injections of foreign DNA into the pregnant mother. Two jaundiced female CNH rats were successfully mated with a jaundiced CNH male. Since both parents were known to be homozygous recessives, all offspring produced could be expected to be jaundiced. One female rat was given 10 daily subcutaneous injections of Addis-Slonaker DNA during the first 10 days after insemination, a total dose of 300 mg DNA; she produced a litter of 5 rats, all of which were jaundiced. All 5 failed to thrive and died early. The oldest survivor died at the age of 4 weeks jaundiced and ataxic. The second female was given 10 daily intraperitoneal injections of Addis-Slonaker DNA during the first 10 days after insemination, a total dose of 174 mg DNA; she produced a litter of 13 rats, all of which were jaundiced. Three of these rats survived to the age of 6 weeks, at which time their serum bilirubin levels varied between 6 and 7 mg/100 ml, levels typical of untreated jaundiced rats in the colony.

Discussion. We have not been able to produce somatic or genetic transformations with DNA in the rat. It is possible that the biological activity of the DNA was destroyed during the isolation process. This seems unlikely in view of the mild chemical procedure used. The amount of DNA injected into these rats, in relation to body weight, was approximately 10 times as great as that given to ducks by Benoit *et al.* (personal communication). A likely explanation for the failure is that DNA when injected into a mammal fails to reach the interior of cells, where its action might take place. The injected DNA would have to cross several cell membranes, as well as to be carried in the blood stream to sites distant from point of injection. During this process it would be subject to destruction by naturally occurring deoxyribonuclease. There is a vast difference here from the situation where single bacterial cells are bathed in a medium containing foreign DNA, and there is only one cell membrane to cross. The possibility also exists that many cells of a higher organism must be transformed genetically to produce an observable somatic effect. In bacterial transformations, only a fraction varying between 0.01% and 15% of the cells present are permanently modified by foreign DNA. If only 1 in 10,000 skin cells were changed so as to produce melanin in an albino rat treated with DNA derived from a pigmented rat, it might not be possible to detect such a minute change by simple observation. In the case of rats that lack glucuronyl transferase in the liver, it was hoped that, if even a small fraction of the hepatic cells could be changed so as to produce the missing enzyme, bilirubin could be cleared from the serum to some extent. Since this failed to occur, it is probable that the injected normal DNA had no effect in altering the abnormal liver cells.

Summary. DNA derived from black and white rats, on injection into newborn albino rats, failed to produce pigmentary changes in the hair and the eyes. Similarly, DNA derived from normal rats failed to correct a genetically determined absence of glucuronyl transferase in newborn jaundiced rats.

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Influence of 6-Deoxy-6-Fluoroglucose on Glucose Utilization in Rat Epididymal Adipose Tissue and Rat Diaphragm.* (24477)

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The compound 2-deoxyglucose (2-DOG) has been shown to inhibit glucose metabolism as early as the hexokinase step(1). However, one difficulty which has been experienced in studies with this compound is a result of a lack of specificity in its blocking action. Thus it is known that 2-DOG blocks glucose metabolism at the isomerase step(2) as well as the hexokinase step and other sites of action of this drug remain as distinct possibilities (2,3). 6-Deoxy-6-fluoroglucose (6-FG) is a glucose analogue which may circumvent the difficulty of multiplicity of action. Because of the absence of a hydroxyl group in the 6 position, the sugar obviously cannot be phosphorylated by hexokinase. Thus it would appear that if 6-FG does competitively inhibit glucose metabolism in mammalian tissues it should do so prior to or at the hexokinase step. Studies of 6-FG action in rat kidney tissues have indicated that this compound is indeed a competitive inhibitor of glucose utilization (4). Moreover, it appears that 6-FG does inhibit at or prior to the hexokinase step in rat kidney slices. A surprising feature of the study with rat kidney tissue has been the anomalous effect of an increased concentration of 6-FG on glucose oxidation. At low 6-FG concentrations a true competitive response to increasing concentrations of the fluoro derivative is observed. At high 6-FG concentrations, however, a plateau is observed in the curve of inhibition of glucose oxidation over a wide range of 6-FG values. These data have been interpreted to suggest the presence of an alternate pathway of glucose metabolism before the hexokinase step. The object of the studies reported here is to determine whether the inhibitory pattern observed with rat kidney tissues is applicable to other mammalian tissues.

Material and methods. The procedures employed in the preparation and incubation of rat adipose tissues and isolated rat diaphragms have been previously described(5,6). When used as substrates concentrations of glucose and acetate were maintained at 0.01M in the incubation medium. 2-DOG was obtained from Nutritional Biochemicals Corp., Cleveland, O. 6-FG was prepared according to the procedure of Helferich *et al.*(7,8) as revised by Blakley(9).

Results. The data of Table I show that fluoride ion is an effective inhibitor of oxidation of glucose and acetate in rat epididymal

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