The metabolic origin of AICHR is not clear. One possibility is that this compound is synthesized from homocysteine by reaction with AIC riboside when tissue concentrations of homocysteine are greatly increased. It is reasonable to assume that such a reaction, if it occurs, must be an enzymatic one. This assumption is rendered more valid by the observation that a normal subject (T.L.P.) who was fed a large quantity of DL-homocystine excreted large amounts of D-homocystine in urine over a 24-hour period, yet did not excrete AICHR. AIC ribotide is a normal intermediate in the biosynthesis of purines and can be dephosphorylated to AIC riboside. The mammalian liver enzyme which condenses adenosine and homocysteine to *S*-adenosylhomocysteine is thought to be quite specific with respect to both substrates (*12*), but the possibility that this enzyme, or an analogous condensing enzyme, might react with AIC riboside as well as with adenosine must be considered.

In fact, a high concentration of homocysteine in tissues might itself favor the accumulation of AIC riboside and thus promote condensation of the latter with homocysteine, if this hypothetical pathway is indeed operative. Warren *et al.* (*13*) have shown that the two biosynthetic reactions in purine synthesis leading from AIC ribotide to 5-formamido-4-imidazolecarboxamide ribotide and then to inosinic acid are reversible in vitro. These investigators found that the conversion of inosinic acid back to AIC ribotide by the liver enzymes which normally synthesize inosinic acid required a reduced folic acid compound, potassium ions, and a reducing substance. Homocysteine was by far the most effective reducing substance tested. It stimulated this reaction, in addition to its effect as a reducing substance, by a mechanism which was unexplained and which might be of importance for the same reaction sequence occurring in vivo when the concentrations of homocysteine in the tissues rise.

A second possibility for the metabolic origin of AICHR is that this compound arises from the degradation of *S*-adenosylhomocysteine. The latter might accumulate in the tissues in homocystinuria, being only one step further removed than homocysteine from the enzymatic block in methionine catabolism. However, we have not yet succeeded in identifying *S*-adenosylhomocysteine in the urine of homocystinurics. The only known mammalian pathway for the degradation of adenine and other purines results in the formation of uric acid and allantoin. Opening the purine ring to form AICHR from *S*-adenosylhomocysteine would imply the existence of a presently unknown alternate route for purine degradation. A theoretically possible degradative pathway from *S*-adenosylhomocysteine to AICHR might involve, first, deamination to *S*-inosylhomocysteine and then, facilitated by excessive homocysteine, an opening of the purine ring and removal of a formyl group by the same enzymes which have been shown to convert inosinic acid to AIC ribotide in vitro (*13*).

Homocystinuria, like other inborn errors of metabolism, may provide clues which lead to the eventual discovery of interesting new biochemical pathways in man. The compound which we have tentatively identified appears to involve the metabolism of purines as well as that of methionine, and the precise manner in which it is formed needs to be determined. It also will be important to explore what part, if any, AICHR and other still unidentified homocysteine metabolites play in producing the pathological manifestations of homocystinuria.

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Retention of Potential to Differentiate in Long-Term Cultures of Tooth Germs

Abstract. *When tooth germs derived from 14-day mouse embryos were cultured on gelatin sponges in vitro for 37 days, they lost their characteristic morphology, appearing as a layer of undifferentiated epithelium on the sponge surface, with the mesenchymal cells scattered throughout the interstices. These cultures were then transplanted subcutaneously into isologous, newborn recipients and, over a period of 56 days, developed into incisor teeth that were almost perfect in shape and structure.*

In experiments primarily designed to investigate viral oncogenesis in vitro, a series of control, uninfected organ cultures were set up as follows.

Mandibular and maxillary incisor tooth buds were dissected under sterile conditions from 14-day mouse embryos (C3H/Bi) and placed on gelatin sponges in Leighton tubes (*1*), five tooth buds to each tube. The medium used was Eagle's minimal essential medium (*2*) supplemented with calf serum (10 percent); the medium was changed every 2nd day; in all 23 tubes were set up.

Ten of the sponges were fixed in a Zenker-formalin mixture, embedded in paraffin, serially sectioned, and stained with hematoxylin and eosin after 7, 10, 14, 21, 28, 42, and 50 days in vitro. The remaining 13 sponges were removed from the Leighton tubes after 37 days in vitro, cut into small pieces, and transferred to 38 newborn syngenic recipients by a fine pipette introduced through an incision at the nape of the neck and passed down to the base of the tail, where the fragments were gently deposited.

Seven of the host mice died early owing to the effects of the operation or to maternal cannibalism, but 31 survivid, and these were killed after 8 weeks.

The incisor tooth buds from 14-day mouse embryos are at the early

cap stage before morphodifferentiation of the individual types of odontogenic cells has begun. After 7 days in organ culture, ameloblasts, outer enamel epithelium, and odontoblasts could be seen; and the enamel organ had progressed to the bell stage of development. In a few cases, a thin layer of calcified tissue had been deposited. By 10 days no further differentiation had occurred, and in some cases squamous metaplasia of epithelium with keratin production had taken place. By this time, also, the mesenchymal cells had invaded the interstices of the gelatin sponge and spread out in all directions. By 14 days the epithelial cells had lost their resemblance to ameloblasts but still retained the outline form of a tooth germ. However the cells of the dentine papilla had undergone necrosis. Mesenchymal cells were by this stage widely scattered throughout the sponge. By 21 days the resemblance to a tooth germ had been lost completely. The epithelial cells either lay in clumps or had spread out over the surface of the sponge. Squamous metaplasia with a little keratin formation could be seen here and there, but in general the cells were undifferentiated and could only be recognized by their cohesiveness, size, and tinctorial characteristics. Individual cell necrosis became an obvious feature in that the cellular debris produced remained *in situ*. The position originally occupied in the sponge by the dentine papilla remained recognizably present as an area of homogeneous eosinophilic tissue debris in a rounded focus of lysed gelatin (Fig. 1).

These appearances were essentially the same in the cultures examined between 21 and 50 days, although the small masses of keratin became larger with time. As no exceptions to these observations were seen, it is presumed that such were the appearances of the cultures transplanted after 37 days in vitro.

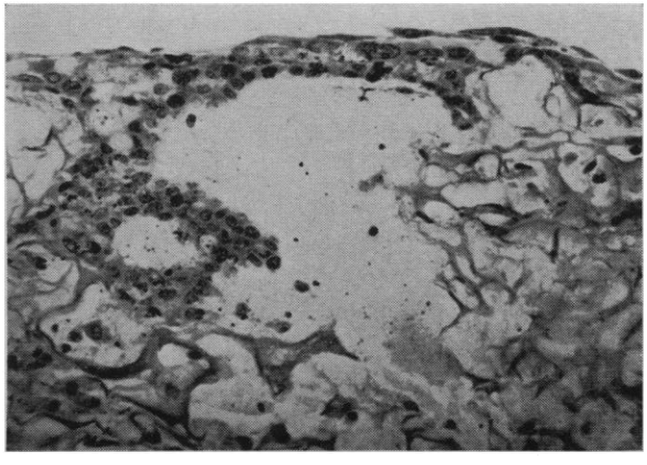
The recipients of the transplanted cultures were killed with ether after 56 days. The dorsal skin was reflected and, with a dissecting microscope when necessary, a careful search was made for the transplanted tissue. In 16 of the 31 mice, incisor teeth were found lying in the subcutaneous tissues around the base of the tail. These were of normal appearance, being curved and having pigmented enamel. Blood vessels entering the open apex of the teeth could be seen macroscopically. In some of the hosts two, three, and even four teeth could be found. All the transplant tissue was dissected out, fixed in the Zenker-formalin mixture, decalcified in 5 percent formic acid, serially sectioned, and stained with hematoxylin and eosin.

In nine host mice no transplanted tissue could be found, in six the tissue consisted only of small clumps of squamous epithelium in which epidermoid, keratin-filled cysts had formed in five cases, and in the remaining 16, teeth were found. On section (Fig. 2) these teeth were found to consist of normal dental tissues with perfectly formed dentine, predentine, odontoblasts, pulp cells, and cementum. The only abnormalities found were in the enamel organ where the ameloblast layer contained occasional irregularly arranged segments. However, a layer of enamel had been formed, and this had matured normally in that changes from basophilia to eosinophilia and from acid insolubility to complete solubility were observed. A number of the teeth formed were of abnormal shape.

The development of the explanted tooth buds found in the first 14 days of this experiment is similar to that recorded by a number of other workers (*3*). There are no reports of organ cultures of teeth carried in vitro for longer than this time.

The retention of the potential of cultured cells to differentiate can be tested in a number of ways that have been listed by Grobstein (*4*) as morphological, behavioral, chemical, and developmental. The method used in this study, that of transplanting the explant into an isologous recipient to observe its development, has been chiefly used with cultures of endocrine glands. Using this method for therapeutic purposes, Stone, Owings, and Gey (*5*) successfully transplanted cultures of human parathyroid after 2 to 4 weeks in vitro, and this work has been extended by Gaillard (*6*). Martinovitch (*7*) and Schaberg (*8*) both transplanted 3- to 8-week cultures of anterior hypophysis to hypophysectomized rats and observed that the cultures became differentiated, forming functional glands. The same authors (*7, 8*) describe similar success with transplants of adrenal cortex after 2 to 7 weeks in culture. In all these cases when long-term cultures were transplanted, the cultures had lost their morphological resemblance to the organ from which they had originally been derived and consisted of cells of undifferentiated appearance.

The observations made in this work were similar. For the initial 10 days or so, normal morphodifferentiation of the tooth was seen, albeit at a much slower



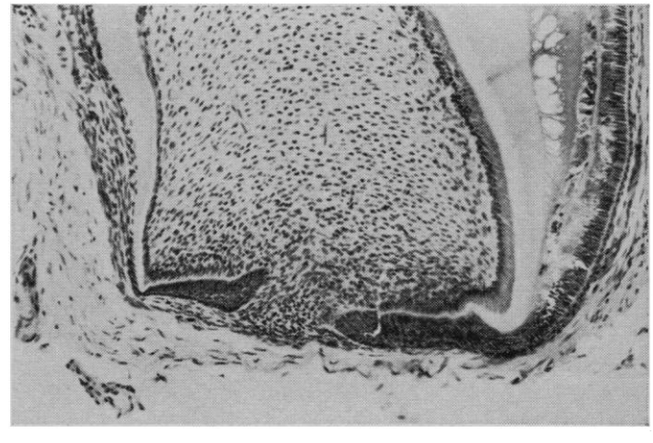


Fig. 1 (left). Section of gelatin sponge showing odontogenic epithelium and mesenchyme after 28 days in vitro. The large space in the sponge was originally occupied by the dentine papilla but this tissue has undergone complete necrosis (hematoxylin and eosin; × 63). Fig. 2 (right). Section of base of incisor that developed from transplanted culture. Almost normal appearances but for irregularity in enamel organ (hematoxylin and eosin; × 16).

rate than that occurring in vivo. Then the central cells in the dentine papillae underwent necrosis, presumably as a result of nutritional insufficiencies. The cells of the enamel organ, over the next 10 days, progressively lost their characteristic appearance and reverted to a morphologically less differentiated type, eventually appearing as a thin layer of stratified epithelium covering the surface of the gelatin sponge.

In view of these extensive changes it was surprising that when this tissue was transplanted into syngenic hosts it had retained the potential to reaggregate into a tooth germ and to produce an almost perfectly formed mouse incisor tooth. It would seem unlikely that there were any epigenetic influences of an inductive nature derived from the host mice on the transplanted tissues, and therefore the potential to develop and differentiate must have been intrinsic.

It was not possible to determine the source of the mesenchyme in the induced teeth, but, as such tissue is known to be mesectodermal in origin (*9*) and specific to some degree as yet not entirely ascertained for tooth formation, it would seem that it must have originated in the transplanted tissue.

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Immunochemical Studies of Submicrosomal Membranes from Liver of Normal and Phenobarbital-Treated Rats

Abstract. *Rabbit antiserums were prepared against three submicrosomal fractions from liver of normal or phenobarbital-treated rats. The two membrane fractions originating from the rough- and smooth-surfaced endoplasmic reticulum were characterized by the same soluble antigens, with the exception of a highly basic component present only in extracts of rough membranes. The third fraction, whose subcellular origin is unknown, was different. It contained at least two typical marker antigens not present in the other fractions. Of eight tissue antigens common for the endoplasmic reticulum, five displayed nonspecific esterase activity. Some of these esterases were also found in other organs, but none was seen in rat serum. Phenobarbital treatment of the rats led to a rise in activity and characteristic changes in the esterase patterns of all these submicrosomal liver fractions.*

Extracts of isolated rat liver microsomes contain freshly synthesized antigens resembling the serum proteins and tissue antigens of unknown significance (*1*). Many of the latter are "cell fraction" as well as "liver" specific and originate mainly from the microsomal membranes of the parenchymal liver cells (*2*). The isolated microsomal fraction of rat liver is heterogenous with regard to subcellular origin and morphology. Isolated "plasma membranes" of rat liver cells (*3*), as well as those of Ehrlich ascites tumor cells (*4*), contain some antigens apparently distinguishing them from other elements of the microsomal fraction. We report an attempt to characterize immunochemically the "rough" and "smooth" membranes of the endoplasmic reticulum of rat liver cells (*5*), isolated from normal or phenobarbital-treated animals. Phenobarbital treatment leads to a specific rise in the activity of certain microsomal enzymes and a concomitant proliferation of the smooth-surfaced membranes of the endoplasmic reticulum of the parenchymal liver cells (*6, 7*).

Rat liver microsomes from starved Sprague-Dawley rats (both sexes) were subfractionated (*8*). Livers from groups

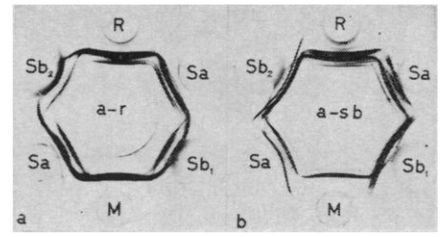

Fig. 1. Unstained precipitin reactions in agar. M, R, Sa, Sb_1, and Sb_2: detergent extracts of total rat liver microsomes, rough, Sa, and two independent preparations of Sb membranes, respectively. Protein concentration of all solutions, 6 to 8 mg/ml; a-r, a-sb: antiserums to rough and Sb membranes, respectively.

of five rats (total, 35 g) were homogenized in ice-cold 0.25*M* sucrose and centrifuged at 10,000*g* for 15 minutes to remove cell debris, nuclei, mitochondria, and lysosomes. Cesium chloride (15 m*M*) was added to the supernatant. It was layered over a 1.3*M* sucrose medium, containing 15m*M* CsCl; the tubes containing these solutions were centrifuged at 250,000*g* for 60 minutes (Christ ultracentrifuge, model Omega). The upper part of the supernatant, including the layer at the gradient boundary, was removed, and $MgCl_2$ was added to it to a final concentration of 10 m*M*. It was then again layered over sucrose (1.15*M*) containing 10 m*M* $MgCl_2$, and centrifuged at 250,000*g* for 30 minutes. The upper part of the supernatant, including the layer at the boundary, was again removed, diluted to 0.25*M* sucrose, and ultracentrifuged once more. In this way, three different fractions were prepared: (i) "rough" membranes, with attached ribosomes, which sediment in the presence of Cs^+ (first ultracentrifugal sediment, fraction R); (ii) "smooth" membranes which sediment in the presence of Mg^{2+} but not Cs^+ (second ultracentrifugal sediment, fraction Sa); and (iii) "smooth" membranes which were sedimented neither by Mg^{2+} nor by Cs^+ (third ultracentrifugal sediment, fraction Sb). Electron microscopical, chemical, and enzymatic studies have provided good evidence that the first and the second sediments originate from the rough- and smooth-surfaced parts of the endoplasmic reticulum, respectively. The origin of fraction Sb is uncertain (*8*). The three types of membrane fractions were injected into groups of four rabbits each. Each rabbit received three intramuscular injections, given at 3-week intervals, of 5 to 10 mg of pro-