sidered subspecies K of their respective species. *Sigmodon medius* and *S. minor* from Arizona are considered subspecies A of their respective species.

Although there is not now any absolute method to allow exact correlation of the Kansas deposits with the Arizona deposits, published and unpublished records of fossil vertebrates from these localities, plus the morphological similarity of *Sigmodon medius* from Kansas to *S. medius* from Arizona, indicate a Blancan date (or dates; Benson Ranch is probably early Blancan, whereas the Tusker Local Fauna is possibly latest Blancan) for deposits in Arizona containing *S. medius*. The Curtis Ranch Local Fauna, containing *S. minor*, must be younger than Blancan, and I tentatively correlate it with the Borchers Local Fauna from Kansas, which also includes *S. minor* (Fig. 1).

The immediate questions that bear on Coon's theory of the origin of human races are these: (i) did *Sigmodon minor* evolve both in Arizona and in Kansas from populations of *S. medius* in each state, or (ii) did *S. minor* evolve in a single area (perhaps neither state) and disperse to Kansas and Arizona? If *S. minor* originated as in case i, then we must find characteristics shared in common by *S. medius* and *S. minor* in Kansas that are distinct from those in the same evolving lineage in Arizona.

Sigmodon medius from Arizona and *S. medius* from Kansas demonstrate almost the same dental pattern. The dental pattern of *S. minor* is different from that of *S. medius*, but the pattern in *S. minor* from Kansas is the same as that of *S. minor* from Arizona. The morphometric response of the lower dentition and mandible within each species from the two states is, however, characteristically different.

Measurements of the mandibles and lower dentitions are graphically portrayed (Figs. 2 and 3) by the method presented originally by Simpson (*13*). When I first made a composite graph (Fig. 2), it was apparent that three groups were represented, but the variation in certain portions of the plots, notably in the region of the mandibular alveolar (MA) length and the length of the M_1, was unusual. When the ratio diagrams for the species were plotted by state (Fig. 3), a state-specific pattern was evident. For the measurements plotted in Fig. 3, it is clear that within each state *Sigmodon minor* differs from *S. medius* solely in size; between states there is an obvious morphometric dichotomy. Subspecies K of *S. medius* differs from subspecies A of *S. medius* in having an absolutely greater average MA length. The same relationship holds true for subspecies K versus subspecies A of *S. minor*. The cause of this increased MA length in the Kansas subspecies has not yet been determined, but it must be related to thickening of mandibular bone, to increased anterior-posterior spacing of the teeth, or to both features.

These differences may be interpreted as in situ evolution (that is, in the same general area but not necessarily in the same square mile), subspecies K of *Sigmodon medius* giving rise to subspecies K of *S. minor*; subspecies A of *S. medius* giving rise to subspecies A of *S. minor*. This evolutionary pattern is analogous to the pattern proposed by Coon, in which the *medius* grade cotton rat equals the *erectus* grade hominid, the *minor* grade cotton rat equals the *sapiens* grade hominid, and subspecies A and K of both *Sigmodon* species are equatable to any two subspecies of *erectus* and their *sapiens* descendants.

Although the pattern demonstrated above fulfills the major criterion for evolution in case i, case ii evolution remains equally as probable. As shown in Fig. 1, *Sigmodon* is absent from the Cudahy Fauna of Kansas (*14*). This fauna contains an array of rootless-cheek-toothed microtine rodents, which apparently replaced *Sigmodon* in the Meade Basin during latest Kansan time. The Cudahy Fauna has also been recognized in northern Texas (*15*), and here too rootless-cheek-toothed microtines appear. When *Sigmodon* is seen again in the Meade Basin (in the Borchers Local Fauna) it is as the very microtine-like *S. minor*. It is clear then that *S. minor* evolved from *S. medius* in some geographical area other than the stratigraphic zone representing the Cudahy Local Fauna. As such, it is conceivable that *S. minor* was derived from a single population of *S. medius*, perhaps in northern Mexico, and that *S. minor* subsequently moved back to both Arizona and Kansas. Given this pattern, the resemblance between *S. minor* and *S. medius* from the same state would by definition be considered parallel evolution. It should be noted, however, that a *Sigmodon* species is absent also from the Dixon and Deer Park Local Faunas of Kansas and yet appears again unchanged from the Rexroad *S. medius* in the Sanders Local Fauna (Fig. 1). Thus it remains possible that *S. medius* was not displaced far south, or at any rate that the Kansas and Arizona *S. medius* populations retained their subspecific (racial) independence as that species in Kansas and Arizona passed the *minor* grade threshold.

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Carbon Dioxide Fixation by Mouse Embryos prior to Implantation

Abstract. *Mouse embryos in the stage of development prior to implantation were cultured in vitro in a medium that contained radioactive bicarbonate. The radioactivity was incorporated into the proteins and nucleic acids that were acid soluble. Uptake of radioactivity occurred into protein in the unfertilized ovum and was highest in all fractions in the early blastocyst stage. No incorporation was detected in the lipid fraction.*

The stages of mammalian embryos prior to implantation develop within the secretions of the oviduct and uterus. Moreover, there is evidence that these early stages are nutritionally dependent on maternal secretions (*1*). One component of secretions of the oviduct, present in considerable concentration, is bicarbonate (*2*); this substance is believed to be an essential component of culture mediums for mouse embryos (*1*). On the basis of these facts, Biggers *et al.* suggested that carbon dioxide fixation occurs during the cleavage stages

of the fertilized mouse ovum (*1*). We have, therefore, exposed mouse embryos in various stages of development to [C^{14}]$NaHCO_3$, and have shown that CO_2 fixation occurs at all stages, and that the labeled carbon participates in the synthesis of macromolecules.

Mouse embryos were collected from superovulated 8-week-old mice. Female mice received 10 international units (I.U.) of pregnant mare serum (Equinex, Ayerst) followed 48 hours later by 10 I.U. of human chorionic gonadotropin (HCG, Ayerst). With the exception of the mice from which the unfertilized ova were obtained, all females were then caged with males. The presence of the vaginal plug indicated copulation. The unfertilized and fertilized ova were obtained on the day after injection of HCG (day 1 of pregnancy), the two-cell stages were obtained on day 2 of pregnancy, the eight-cell stages on day 3, and morula and early blastocysts on day 4 and the late blastocysts on day 5.

Embryos were flushed from the reproductive tract and washed twice in a simple culture medium (*3*). Up to 200 embryos were then transferred to a glass centrifuge tube containing 0.02 ml of culture medium that contained C^{14}-labeled $NaHCO_3$; the tube was then covered with mineral oil. The tubes were gassed with 5 percent CO_2 in air and incubated at 37°C for 4 hours. After incubation the embryos were removed from the culture tubes and washed by centrifugation through a column of aqueous sucrose (*4*). The centrifuged embryos were transferred through two changes of nonlabeled medium in embryological watch glasses, and the recovered embryos were counted. The embryos were then placed on a piece of filter paper (Whatman No. 1). A volume of washing medium, equal to that used to suspend the embryos during transfer to the filter paper, was placed on another piece of filter paper and served as a control. The filter papers were dried and stored up to 1 week at 5°C before the radioactivity was counted. Any labile C^{14} present as bicarbonate and carbamino compounds should have been eliminated by the washing and drying procedures. Radioactivity was determined by the procedure of Mans and Novelli (*5*) as modified by Tyler (*6*). The embryos were treated with 10 percent trichloroacetic acid, with boiling ethanol and then a mixture of ethanol and ether, with ribonuclease (Sigma, 1 mg/ml in water at 37°C), and with 5 percent hot 90°C) trichloroacetic acid to remove the acid-soluble material, lipids, RNA, and DNA, respectively (*7*).

Table 1. Radioactivity (counts per minute per 100 embryos) incorporated into embryos during a 4-hour incubation at 37°C in the presence of C^{14}-labeled $NaHCO_3$. Values are determined by the difference in the amount of radioactivity present before and after each extraction procedure. All counts were corrected for background and for contamination by subtracting the radioactivity present on the control filter papers from that of the corresponding filter papers that contained the embryos. There was no loss of radioactivity from the filter paper into the scintillation fluid. Specific activity of incubation medium was 1.6 μc/ml and 17.7 μc/ml in replicates No. 1 and No. 2, respectively.

Stage	Fraction					
	Acid soluble	Lipid	Nucleic acid	RNA	DNA	Protein
			Replicate 1			
One-cell, unfertilized	35.4	0	0			6.3
One-cell, fertilized	40.7	0	0			7.1
Two-cell	26.6	0	1.5			1.5
Morula	44.3	0	14.2			13.3
Early blastocyst	37.8	0	15.9			13.2
Late blastocyst	21.8	0	17.4			8.8
			Replicate 2			
Control*	5.2	0		0	0	3.1
One-cell, unfertilized	141.5	0		0	4.2	26.9
One-cell, fertilized	77.7	0		1.7	0.8	24.1
Two-cell	273.5	0		5.8	3.2	14.2
Eight-cell	652.9	0		105.2	142.0	138.0
Morula	787.4	0		163.9	351.8	423.8
Early blastocyst	1067.4	0		234.0	585.4	479.4
Late blastocyst	516.2	0		132.4	244.2	372.1

* Treated, then incubated in 1 percent formalin.

Two replicates were carried out with different samples of labeled sodium bicarbonate. The differences between the two replicates are due to the differences in specific activities of the two samples. Replicate No. 1 did not include the eight-cell stage, and only total nucleic acid content was measured. Replicate No. 2 included the eight-cell stage, and DNA and RNA were analyzed separately (Table 1).

Carbon dioxide fixation occurs in all stages of the mouse embryo prior to implantation. In order to demonstrate that the incorporation of radioactivity depends on cellular transport or metabolism, groups of embryos were first incubated for a short period in a culture medium that contained 1 percent formalin, and then subsequently incubated in the medium with labeled bicarbonate. No accumulation of radioactivity occurred under these conditions. Thus, the combined evidence strongly suggests that a mammalian embryo which develops under viviparous conditions can be added to the list of nonmammalian oviparous forms which fix carbon dioxide in early development (*8*). That CO_2 fixation occurs in a variety of embryos is not surprising since the phenomenon seems to be an essential process in heterotrophic cells (*9*). Its roles are multiple. These include the formation of key metabolic intermediates, such as oxaloacetate; substances essential for growth, such as aspartate and purine bases; and catalysis of certain sequences of enzyme reactions. The results also demonstrate that the C^{14} participates in the formation of nucleic acids and protein, although the degree of incorporation varies with the stage of development. No apparent incorporation of radioactivity into the lipid fraction occurred during any stage of development. Protein synthesis occurs at all stages of development and in the unfertilized ovum of the mouse. These observations confirm the qualitative autoradiographic observations of Mintz (*10*) on the developmental stages of the mouse after fertilization, and the quantitative observations of Monesi and Salfi (*11*) who showed that DL-[4,5-H^3]lysine and L-[4,5-H^3]leucine were incorporated into the unfertilized mouse ovum and into the embryo at all stages prior to implantation.

The incorporation of C^{14} from bicarbonate into protein seems to follow the pattern of uptake of DL-[4,5-H^3]lysine rather than that of L-[4,5-H^3]leucine (*11*). A significant amount of synthesis is observed in unfertilized and fertilized ova, and is considerably less at the two-cell stage. At the eight-cell stage a marked increase in the degree of incorporation occurs. Weitlauf and Greenwald (*12*), however, found no uptake of [^{35}S]methionine by two-cell mouse embryos in vivo, and they concluded that no protein synthesis occurs at this stage

of development. However, it is possible that, like leucine, little uptake of methionine occurs at this time, and thus, their method is not sufficiently sensitive to detect the small amount of protein synthesis demonstrated by CO_2 fixation and the incorporation of lysine.

An important question concerns the role that CO_2 fixation plays in the normal development of embryos in their natural physiological environment. All embryos are exposed to CO_2 in early development, but the environmental concentrations vary considerably. Oviparous forms develop in an aquatic environment, where CO_2 exists as bicarbonate and carbonate ions, carbonic acid, and undissociated molecules of CO_2. The total CO_2 content of seawater, for example, is approximately 2.0 mmole per liter (*13*).

Analyses of the secretions of the oviducts in the rabbit and sheep show that much higher concentrations of bicarbonate occur. These concentrations are close to those found in blood (*14*) and are about 10 to 15 times higher than in seawater. Thus, at the time of fertilization and early development, mammalian embryos, unlike aquatic forms, are exposed to an environment that is rich in bicarbonate, thereby providing ample opportunity for CO_2 fixation. The known importance of bicarbonate in mediums used to culture mouse embryos at stages prior to implantation suggests that CO_2 is essential for early development in this species. Moreover, in the sheep, the bicarbonate content of the oviduct fluid is higher during estrus than it is during diestrus, and it is elevated in ovariectomized ewes after the injection of 17β-estradiol (*2*). Perhaps, during the evolution of viviparity in mammals, a hormonally controlled mechanism for enhancing the content of bicarbonate in the oviductal secretions has been selected.

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Hyperbaric Oxygen: Effects on Metabolism and Ionic Movement in Cerebral Cortex Slices

Abstract. *The incubation of slices of cerebral cortex under hyperbaric oxygen pressures from 1 to 10 atmospheres in the presence of radioactive glucose, pyruvate, succinate, fumarate,* L-*glutamate, and gamma-aminobutyric acid causes a marked diminution of tissue oxidative reactions. There is a simultaneous decrease in phosphocreatine and adenosine triphosphate, and a reduction of the apparent intracellular ionic gradients. The increase of lipid peroxides, measured directly, is attributed to the toxic effects of hyperbaric oxygen.*

Exposure of man and animals to oxygen at high pressure induces generalized convulsions similar to epileptic fits (*1, 2*). In man, convulsions occur after a latent period which is inversely proportional to the partial pressure of oxygen (*3*). Also, hyperbaric oxygen has an inhibiting action on numerous enzymes (*4*) and lowers the oxygen uptake of brain slices and homogenates (*5*). The onset of convulsions appears to occur before significant alteration of metabolic reactions (*1, 4, 6*). We have attempted to determine whether hyperbaric oxygen alters oxygen uptake in guinea pig cerebral cortex slices incubated in Krebs-Ringer glucose saline solution or in the presence of various substrates. The concentrations of glycogen, inorganic phosphate, phosphocreatine, adenosine triphosphate, K^+, and Na^+ were studied with glucose as the oxidizable substrate. Formation of lipid peroxides, thought to be connected with oxygen toxicity, was measured by direct chemical analysis, whereas formerly it was estimated indirectly from cerebral peroxidized unsaturated fatty acids (*7*).

Cerebral cortex slices (0.35 mm thick, weighing 60 to 90 mg) from guinea pigs were cut dry with a bow cutter and incubated in Krebs-Ringer glucose saline solution [buffered with tris(hydroxymethyl)aminomethane] (*8*). All incubations were carried out at 37°C in 5 ml

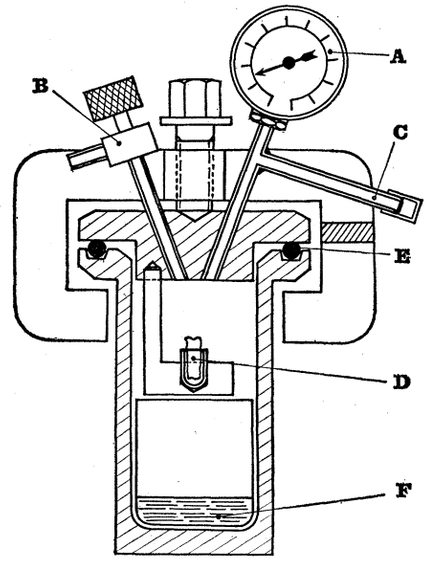

Fig. 1. Sealed cylinder used for the incubation of slices under high oxygen pressure. *A*, Manometer; *B*, valve; *C*, valve of car-tire, for gas entry; *D*, paper saturated with KOH; *E*, rubber rim; *F*, saline (5 ml) in a polythene container. Total volume of the sealed cylinder is 200 ml.