37°C into subzero air. But in warmer climates vapor is probably not an important part of the spout. Our observations of whales in Baja California calving lagoons, where the air temperature was usually between 10° and 20°C, show the following.

1) A spout does not appear every time they breathe. When animals float quietly and do not submerge between breaths a spout is not produced, or is weakly evident.

2) Small spouts are sometimes seen from slowly swimming animals whose nostrils are apparently wholly above the sea. The water source here may be a result of the mechanism of the nostrils closing. For example, the bottlenose porpoise, *Tursiops truncatus*, often submerges while the blowhole is still open, and water leaks down into a wedge-shaped cavity that is closed by nasal plugs at the superior bony nares (*2*). If the gray whale is similarly lax in closure of its blowholes (nostrils), sufficient water could be trapped to produce the smaller spouts.

3) Serial photographs of gray whales show that the blow often begins before the nostrils break the surface. Fully developed spouts then occur and they are dense at the nostrils, containing large drops or even ragged sheets of water (Fig. 1). Upward in the spout the water is atomized to a mist. Theoretically, if the spout were condensation due to rapid expansion and cooling of expired gases, the major effect should occur high in the spout and not at the nostrils.

In sum, the gray whale's spout is composed mainly of seawater, and its magnitude is controlled by the way in which the whale begins and ends its respiratory cycle.

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Biogenesis of Erythropoietin: Evidence for Pro-Erythropoietin in a Subcellular Fraction of Kidney

Abstract. *The light mitochondrial fraction of hypoxic rodent kidneys, called the renal erythropoietic factor, contains erythropoietin in a pro, or inactive form. Erythropoietin is released from this inactive form when the renal erythropoietic factor is incubated with normal serum. The biogenesis of erythropoietin possibly involves a system in kidney reminiscent of the proinsulin-insulin system in pancreas.*

The biogenesis of erythropoietin (Ep), although extensively investigated, has not yet been elucidated. Gordon *et al.* (*1*) presented extensive evidence indicating that under both normal and hypoxic conditions, the biogenesis involves interaction between a renal erythropoietic factor (REF), also called erythrogenin, and a normal serum component. The REF is found mainly in the light mitochondrial fraction of kidney homogenates, which is largely composed of lysosomes. These findings suggest alternative mechanisms for the biogenesis of Ep (*2*). Thus, it may be postulated that the REF is a renal enzyme that activates a serum substrate, possibly derived from the liver (*1*); only suggestive, rather than conclusive, evidence favoring this hypothesis was presented (*3*). In addition, in accordance with the original concept of Kuratowska (*4*), the possibility should be considered that the REF contains a pro-Ep factor which is activated by normal serum. This hypothesis, however, is unsubstantiated by experimental evidence. Finally, the possibility cannot be excluded that the REF contains both Ep and an inhibitor of Ep that is not chemically linked to Ep and would be rendered inactive by a serum factor, thus unmasking the Ep activity.

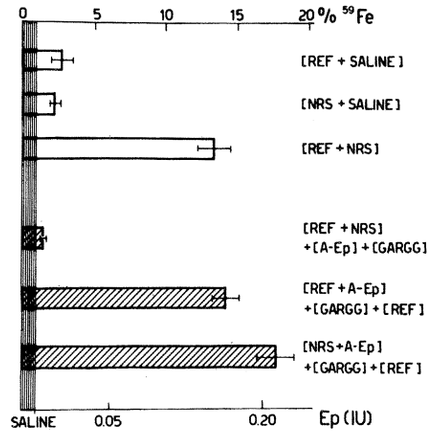
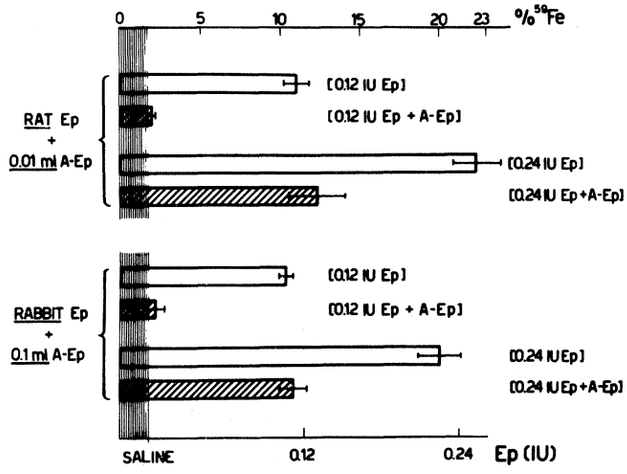


Fig. 1. REF plus NRS: incubation with A-Ep and GARGG. Percent of ^{59}Fe incorporated into red blood cells (means ± S.E.M.) and corresponding international units of Ep in assay mice receiving (first three bars from the top) rat REF plus saline, normal rat serum (NRS) plus saline, or REF plus NRS, respectively. The other three vessels (REF plus NRS; REF; NRS) were incubated with antiserum to Ep (A-Ep) and then GARGG; REF and NRS vessels were subsequently incubated with NRS or REF, respectively. All mixtures were finally injected in assay mice.

Our results indicate that a pro-Ep molecule in the REF is rendered active by incubation with normal serum. We therefore suggest that the biogenesis of Ep is mediated by a system consisting of pro-Ep and Ep that is reminiscent of the proinsulin-insulin complex in pancreas (*5*).

Female CF 1 mice (20 to 25 g), male Wistar rats (150 to 200 g), and young, albino male rabbits were maintained with standard laboratory diets and given free access to tap water. The REF was prepared (*6*) from kidneys of hypoxia-exposed rats or rabbits (0.42 atm of air for 18 hours). Serum from normal rats or rabbits was dialyzed against 100 volumes of the disodium salt of EDTA (0.005*M*) for 24 hours at 4°C. The material was redialyzed against deionized water (100 volumes) for 24 hours and the dialyzate, called rat or rabbit normal serum, was frozen at −20°C until needed. In the incubation procedures, equal volumes of REF and normal serum were incubated for 45 minutes at 37°C in a water bath incubator with constant shaking. In control vessels, REF or normal serum was incubated with equal volumes of physiological saline under the same conditions. In experiments with rabbit antiserum to Ep and goat antiserum to rabbit γ-globulin (GARGG), (Antibodies Inc.) (*7, 8*), the vessels (REF, normal serum, or REF and normal serum, or both) were then incubated with an appropriate amount of antiserum to Ep for 20 minutes under the above conditions. Thereafter, in order to eliminate a possible excess of antiserum to Ep, GARGG was added to the vessels for 15 minutes under the same conditions. The mixture was finally centrifuged at 2000 rev/min for 15 minutes and the sediment was discarded. Our antiserum to Ep was obtained by a modification (*7*) of the procedure of Schooley and Garcia (*9*). One milliliter of the antiserum neutralizes up to 125 international units (I.U.) of human Ep (*10*). The appropriate amount of GARGG had been ascertained by testing against known quantities of antiserum to Ep. This control procedure was always re-



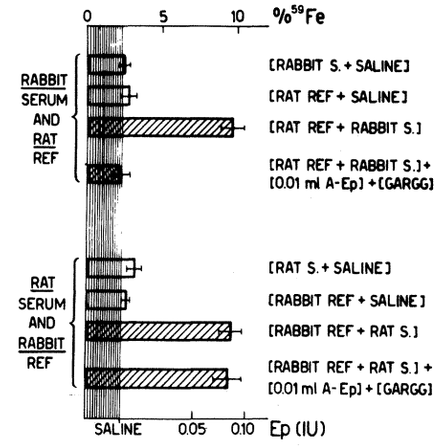


Fig. 2 (left). Rabbit antiserum to human Ep: neutralization of rat or rabbit Ep. Incubation of either 0.01 or 0.1 ml of rabbit antiserum to human urinary Ep respectively with rat or rabbit serum Ep (0.12 or 0.24 I.U. of both rat and rabbit Ep). The incubation was carried out for 30 minutes at 37°C in a water bath, with constant shaking. The precipitate after centrifugation (2000 rev/min for 15 minutes) was discarded and the supernatant was injected in assay mice. The erythropoietic activity was evaluated on the basis of percentage of ^{59}Fe incorporation (into red blood cells) values (means ± S.E.M.) or the corresponding international units of Ep. The antiserum to Ep was further titrated against rat Ep. Thus, 0.20 I.U. of rat Ep, incubated according to the above procedure with 0.005, 0.01 or 0.02 ml of antiserum to Ep, showed the following erythropoietic activity in assay mice: 11.02 ± 1.33, 8.76 ± 0.89, 3.65 ± 0.54; 0.20 I.U. of rat Ep + 0.02 ml of normal rabbit serum: 13.85 ± 0.74. Standards: saline, 2.53 ± 0.18; 0.05 I.U. of Ep, 5.26 ± 0.37; 0.20 I.U. of Ep, 12.40 ± 0.71. The erythropoietic activity was evaluated on the basis of the percentage of ^{59}Fe incorporation (means ± S.E.M.). Fig. 3 (right). Rat or rabbit REF plus rabbit or rat serum: neutralization by rabbit antiserum to Ep and GARGG. Incubation of either rabbit normal serum with rat REF, or rat serum with rabbit REF. The mixtures of REF plus serum were further incubated with 0.01 ml of antiserum to Ep (A-Ep) and GARGG. The activity in the final mixtures was evaluated in assay mice, on the basis of the percentage of ^{59}Fe incorporated into red blood cells (means ± S.E.M.) or corresponding international units of Ep. Abbreviations: rabbit S and rat S, normal serum from rabbits and rats, respectively.

peated in experiments involving antiserum to Ep and GARGG. The erythropoietic activity of test materials was assessed in exhypoxic polycythemic mice by a slight modification of a described procedure (*11*). Thus, test materials were injected intraperitoneally on days 3 and 4 after hypoxia, and ^{59}Fe-labeled ferrous citrate was fed intravenously on day 5. A portion (2 ml) of the incubation mixture was injected into each mouse. Results are expressed as the percentage of ^{59}Fe-labeled red blood cells (that is, the percentage of ^{59}Fe incorporated) or as equivalent units of the first or second International Reference Preparation of Ep. We used a minimum of six mice per group.

Figure 1 indicates that the erythropoietic activity in the incubation mixture of rat REF plus normal rat serum is fully abolished after a two-step incubation with antiserum to Ep and GARGG, thus confirming a previous report by Schooley *et al.* (*12*). This suggests that Ep was generated during the incubation period. It is interesting that preliminary incubation of the REF with antiserum to Ep and GARGG did not modify the Ep activity generated by subsequent incubation of the REF with normal serum. This phenomenon precludes the possibility of simultaneous presence in the REF of Ep and an inhibitor that is not linked chemically to Ep and that would be rendered inactive by a serum factor.

Although 0.01 ml of antiserum to Ep neutralized up to 0.12 I.U. of rat serum Ep, a tenfold larger volume of antiserum was required to neutralize an equivalent activity of rabbit serum Ep (Fig. 2). The potency of this antiserum to Ep (international units of Ep neutralized by 1 ml of serum) may thus be estimated in the range of 12 or 1.2 I.U. of antiserum to Ep per milliliter when tested respectively against rat or rabbit Ep. It thus becomes crucial that the 0.08 I.U. of Ep generated after the addition of normal rat serum with rabbit REF was not neutralized by the addition of 0.01 ml of antiserum to Ep and then GARGG (Fig. 3). On the other hand, the equivalent activity generated after the incubation of normal rabbit serum and rat REF was totally neutralized by addition of the same volume of antiserum to Ep and then GARGG (Fig. 3). Equivalent results were observed in two experiments equivalent to the above and in studies involving a similar incubation of these materials (rabbit or rat serum plus rat or rabbit REF, respectively) with antiserum to Ep without further addition of GARGG.

Our experiments indicate that the Ep molecule is present in the REF and not in normal serum. However, both the immunologic determinants and the active site of Ep in the REF are apparently masked by a carrier group, that is, the Ep-carrier complex constitutes a pro-Ep factor. Thus, the Ep-generating activity of the REF is not inhibited by preliminary incubation with antiserum to Ep and GARGG. Furthermore, the REF is, by itself, biologically inactive. However, incubation of the REF with normal serum unmasks both the active site and the immunologic determinants of Ep, thus generating a significant Ep activity which may be neutralized by antiserum to Ep. Although the results are not presented here, several physicochemical procedures (such as prolonged storing and repeated freeze-thawing) may occasionally release active Ep from the pro-Ep complex (the REF) without incubation with normal serum.

Our results do not necessarily contradict the enzyme-substrate hypothesis postulated by Gordon *et al.* (*1*). In this regard, the possibility exists that, in the REF, pro-Ep functions as a substrate for a renal enzyme, although such an enzymatic interaction requires a third component, the serum factor, to generate Ep.

Thus our evidence indicates that the Ep molecule, synthesized by the kidney, is extracted as a pro-Ep factor from the REF and unmasked from the pro-Ep complex by the interaction of the REF with a normal serum component.

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Stages in Adolescent Involvement in Drug Use

Abstract. *Two longitudinal surveys based on random samples of high school students in New York State indicate four stages in the sequence of involvement with drugs: beer or wine, or both; cigarettes or hard liquor; marihuana; and other illicit drugs. The legal drugs are necessary intermediates between nonuse and marihuana. Whereas 27 percent of high school students who smoke and drink progress to marihuana within a 5- to 6-month follow-up period, only 2 percent of those who have not used any legal substance do so. Marihuana, in turn, is a crucial step on the way to other illicit drugs. While 26 percent of marihuana users progress to LSD, amphetamines, or heroin, only 1 percent of nondrug marihuana users and 4 percent of legal drug users do so. This sequence is found in each of the 4 years in high school and in the year after graduation. The reverse sequence holds for regression in drug use.*

Although marihuana is usually considered as the first step in drug use, such a view is both arbitrary and inadequately documented (*1*). Previous attempts to ascertain sequences of drug use over periods of time have been inferential and based either on interrelations in patterns of use at one point in time, or on retrospective reports in which subjects are asked to recall what drugs they used in the past and the order in which they used them (*2*). Direct delineation of sequences of drug use requires prospective longitudinal studies in which the drug use histories of the same individuals are examined over a period of time.

I now present data from two longitudinal surveys based on random samples of high school students in New York State, and I find that drug use does not begin de novo with marihuana, but with legal drugs: beer or wine at first, and cigarettes or hard liquor subsequently. Some of the youths who smoke or drink continue on to use marihuana, and some of the marihuana users progress further to use one or more other illicit drugs. On the basis of these data, I propose a model for involvement in drug use based on a sequence of four well-defined stages: beer or wine; hard liquor or cigarettes; marihuana; and other illicit drugs (*3*).

Data on sequences of use over time were derived from two longitudinal cohorts of adolescents: (i) a two-phase random sample of adolescents representative of public secondary school students in New York State, who were surveyed in their classrooms with the use of structured, self-administered questionnaires, in the fall and spring of one academic year at an interval of 5 to 6 months (N = 5468); and (ii) the senior class members from the same 18 sample schools, who were contacted a third time 5 to 9 months after their graduation from high school (N = 985) (*4*). At each of the three times, adolescents indicated (i) whether they had ever used and (ii) used within the past month, each of 14 legal and illegal substances. At times 2 and 3, adolescents were also asked about the use of each drug during the interval between the current survey and the preceding one.

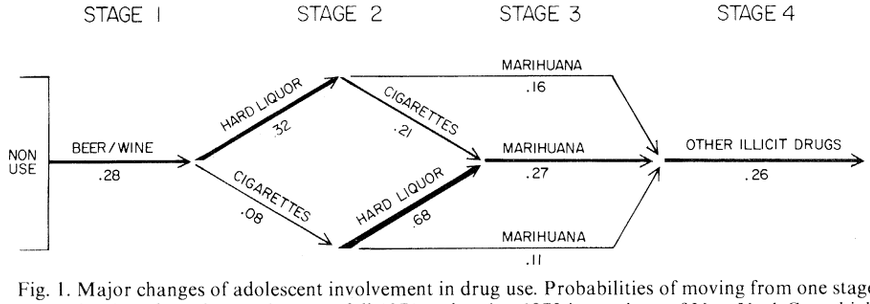

Fig. 1. Major changes of adolescent involvement in drug use. Probabilities of moving from one stage to another based on changes between fall 1971 and spring 1972 in a cohort of New York State high school students, 14 to 18 years old. Youths who started using more than one drug within the follow-up interval were distributed in a sequential order which reproduced the proportions of known exclusive starters of each drug.

The first suggestion of stages in drug use came from the earlier scalogram analyses of data from the first survey of the total high school sample (*5, 6*). The results indicated that adolescent drug use behavior fitted a valid Guttman scale (*5, 6*). The patterns of all the drugs ever used could be arranged according to a well-defined cumulative and one-dimensional hierarchical order with seven steps: (i) nonuse; (ii) legal drugs only (beer, wine, cigarettes, or hard liquor); (iii) cannabis (marihuana, hashish); (iv) pills (ups, downs, tranquilizers); (v) psychedelics (LSD, other psychedelics); (vi) cocaine; and (vii) heroin. Any response that deviates from this order is called an error. Thus, an error occurs when a respondent has used a drug ranked high on the scale (such as heroin), but has not used a lower ranked drug (such as pills). The scale had coefficients of reproducibility of .98 and of scalability of .64 (*7*). The fit of the data with the Guttman scale model implied that youths at any one step have used the drug at that particular level as well as all lower ranked drugs, but they have not used any of the higher ranked drugs. Since these earlier findings were based on data gathered at a particular time, no time order among the usage patterns could be established. Direct evidence for the existence of stages requires longitudinal data.

Although Guttman scaling has been used solely to rank responses at a single time, I have used it here for analyzing movement from one step to another during an interval of time. The power of the approach resides in the fact that Guttman scaling provides, for each respondent, a complete and unambiguous summary of cumulative patterns of drug use up to a particular point in time (or during a specified period). Therefore, it can clearly show subsequent progressions or regressions from these patterns, as well as the extent to which changes follow the cumulative steps specified in the scale, an important criterion for determining the existence of stages in drug use.

In each cohort, the patterns of all the drugs ever used by an adolescent at the time of the initial interview were defined in terms of the seven-step Guttman scale classification described above, and were related to the adolescent's subsequent pattern of use during the follow-up interval. Drug use in the interval was also classified in terms of a Guttman scale, independently of drug use patterns at the initial interview. Results for the total high school cohort (Table 1) are completely rep-