centration of nerve cells *in vivo* and about doubles the normal respiration of brain slices in the presence of glucose. Other incubation flasks contained, in addition, 12.5 mg ethanol, 25 μg acetaldehyde, and 250 μg acetate which correspond to levels of these substances present in blood after an acute dose of ethanol. Flasks were swept with 95% O_2–5% CO_2 before closing and incubated with shaking at 37°C for 90 min. Then 0.5 ml 30% KOH was injected into the center well which contained a small role of filter paper, and 0.2 ml 10*N* H_2SO_4 was tipped into the main body of the flask from the side arm. After shaking the flask and its contents for an additional hour the filter paper and contents of the center well were quantitatively removed and diluted to 10 ml. The capped tubes were allowed to stand for 16 h with occasional shaking at room temperature to assure complete elution of carbonate from the paper. Aliquots were precipitated with $BaCl_2$ containing NH_4Cl in the presence of carrier K_2CO_3 and 'infinitely thick' layers of $BaC^{14}O_3$ were mounted and assayed for radioactivity in a micromil window-equipped flow counter. Sufficient counts were totaled so that the counting error was less than 4% at the 0.05 level of significance. The activity of the glucose-1-C^{14} and glucose-6-C^{14} used in these experiments was determined after complete oxidation to $C^{14}O_2$ by potassium persulfate[6].

Incubations of only 90 min were used in order to minimize any effects due to recycling and randomization of the carbon atoms of glucose, since it has been shown that in mammary tissue the ratio of $C^{14}O_2$ from glucose-1-C^{14} to $C^{14}O_2$ from glucose-6-C^{14} is much higher after 1 h incubation than after 2 and 3 h periods[7]. This precaution, plus the use of K^+ stimulated brain slices, produced more valid experimental conditions than those existing in earlier investigations.

The Table shows that the ratio of $C^{14}O_2$ from the oxidation of glucose-1-C^{14} to that from glucose-6-C^{14} was close to unity for slices from brain cortex in either the presence or absence of ethanol and its metabolites, and hence no evidence for a functioning hexose phosphate oxidative pathway could be demonstrated. KATZ and WOOD[8] have pointed out the difficulties that arise when an attempt is made to calculate from tracer studies the *percentage* of glucose that is metabolized *via* the glycolytic pathway as compared with that *via* the hexose monophosphate pathway. No such attempt was made in this study. The question these experiments were designed to answer was whether there is any evidence that glucose *can be* metabolized by brain *via* the hexose monophosphate shunt under normal conditions or during ethanol intoxication. The results show the answer to be negative, and this reposes the question of the significance of the observable activities of the glucose-6-phosphate and 6-phosphogluconate dehydrogenases in brain. One possible explanation would invoke a carry over of these two enzymes from an embryologically functional phosphogluconate pathway operating at low oxygen tension. The young, but not adult, brain synthesizes its own cholesterol and other lipids *in situ* and this would permit efficient reoxidation of TPNH formed by the dehydrogenases of the phosphogluconate pathway without a requirement for oxygen.

Oxidation of glucose-1-C^{14} and glucose-6-C^{14} by rat brain slices

	No. of rats	% Conversion of labeled glucose to $C^{14}O_2$		$\frac{C^{14}O_2 \text{ from gl-1-}C^{14}}{C^{14}O_2 \text{ from gl-6-}C^{14}}$
		Glucose-1-C^{14}	Glucose-6-C^{14}	
Controls	5	1.79 ± 0.28	1.60 ± 0.06	1.12
Ethanol-treated	4	2.54 ± 0.47	2.08 ± 0.32	1.22

Slices of cerebral cortex (350 mg) incubated with shaking at 37°C for 90 min in presence of 20 mg glucose containing 0.2 μC of either glucose-1-C^{14} or glucose-6-C^{14} in 5 ml Krebs-Ringer bicarbonate containing 0.1*M* KCl. 'Ethanol-treated' preparations contained in addition 12.5 mg ethanol, 25 μg acetaldehyde and 250 μg acetate. Gas phase, 95% O_2–5% CO_2. Results are expressed as means ± standard error.

Résumé. L'auteur a repris la question de l'existence d'une voie oxydative fonctionnelle directe pour la glucose dans le cerveau adulte et constaté qu'elle ne peut être décelée ni dans des conditions normales ni lors d'une intoxication du sujet par l'étanol.

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The Short-term Effects of a Single Dose of Erythropoietin upon Reticulocytes in Starved Rats[1]

Despite the voluminous literature describing the effects of erythropoietin[2–4], its site of action has not been clearly established. Some authors have suggested that erythropoietin acts exclusively upon the stem cell with the resultant production of increased numbers of erythrocytic precursors[5,6], while others have contended that this material may also exert its action by increasing cellular division among nucleated erythrocytes that have advanced well beyond the stem cell stage[3,7]. Measurement of the rapidity of the reticulocyte response should furnish some information as to which of these views is correct. If erythropoietin acts exclusively upon the stem cell, following its injection at least one entire maturation cycle of nucleated red blood cells would have to be completed before there would be any evidence of the delivery of newly-formed reticulocytes into the blood. This would

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entail a relatively slow response that would be expected to take about 48 h[8], assuming that proliferation of erythrocyte precursors would not, in itself, furnish a stimulus for the release of reticulocytes. On the other hand, a demonstration of the rapid appearance of young reticulocytes in the peripheral circulation would indicate that the action of erythropoietin is not restricted to the stem cells. This report is concerned with morphologic and quantitative changes in circulating reticulocytes during the first 24 h following the intraperitoneal injection of erythropoietin into starved rats. It presents evidence to support the hypothesis that erythropoietin can act, at least in part, upon loci other than stem cells.

Methods. Male rats of the Sprague-Dawley strain (Holtzman Co.) weighing 160–175 g were starved for 72 h in order to depress erythropoiesis and were then given an intraperitoneal injection of an erythropoietin preparation. These animals received no food but were allowed water *ad libitum* during the remainder of the experiment. Reticulocyte counts were made at 6, 12, and 24 h after the injection of erythropoietin. The erythropoietin preparation tested consisted of a sheep plasma extract (Armour Lot No. K103124) obtained from animals rendered severely anaemic by phenylhydrazine injections. The extract was dissolved in 0.9% pyrogen-free saline solution shortly before being injected; the dose administered has been expressed in cobalt units[9,10]. Reticulocytes were stained with new methylene blue[11] and were counted randomly using a Miller optical disc[12]. Because of the small numbers of reticulocytes present in starved animals, the equivalent of 20000 to 50000 erythrocytes was examined on each preparation.

Results. Table I summarizes the reticulocyte response to a single injection of erythropoietin. Although no detectable differences in reticulocyte percentages were seen after 6 h, after 12 h the experimental rats had significantly higher percentages of reticulocytes than did their controls ($P < 0.05$), and this increase was even more apparent after 24 h ($P < 0.01$). In addition, the slides were examined for the presence of young reticulocytes which are characterized by a large amount of clumped, stainable reticulum in their cytoplasm. Such cells have been designated as 'grade I' reticulocytes by HEILMEYER and BEGEMANN[13]. These cells, virtually non-existent in the blood of control animals, were seen in two of the experimental animals as early as 12 h after injection and in all of the experimental animals after 24 h. The sparsity of these cells makes their precise quantification technically difficult; in these experiments they never exceeded 0.1% of the total erythrocyte population. In another experiment (Table II) each animal was tested three times for reticulocytes, a single drop of tail vein blood being used for each determination. While the control group exhibited a significant drop in the percentages of circulating reticulocytes as a reflection of the starvation treatment, the erythropoietin-treated animals maintained their reticulocyte level so that 12 h following injection, the experimental animals displayed significantly higher reticulocyte values than did the controls ($P < 0.01$). Furthermore, the appearance of young reticulocytes of the 'grade I' type again were seen as early as 12 h after injection, and all of the animals of this group responded positively in this regard after 24 h. Red blood cell counts done at the conclusion of the experiment revealed no significant differences between experimental and control groups.

Tab. I. Reticulocyte response to a single injection of 30 cobalt units sheep plasma erythropoietin in starved rats

Treatment[a]	h after injection	% Retics (Means ± standard errors)	Young, grade I retics positive response/ total No. of rats
Erythropoietin (6)	6	0.35 ± 0.10	0/6
Control (5)	6	0.26 ± 0.03	0/5
Erythropoietin (6)	12	0.30 ± 0.06[b]	2/6
Control (6)	12	0.12 ± 0.01	0/6
Erythropoietin (4)	24	0.79 ± 0.07[c]	4/4
Control (5)	24	0.17 ± 0.07	0/5

[a] Prepared by Armour Pharmaceutical Co. in cooperation with the University of Chicago for distribution by the U.S.P.H.S. under Research Grant H5393 and kindly furnished by Drs. L. O. JACOBSON and E. GOLDWASSER. This extract appears to be a glycoprotein; its preparation and characterization have been described[9,10]. – No. of animals in parentheses. Control groups received equal amounts of heat-inactivated erythropoietin (dissolved in 0.9% pyrogen-free saline and kept at 95°C for 15 min). Each rat was used for only a single determination.
[b] $P < 0.05$.
[c] $P < 0.01$.

Tab. II. Reticulocyte response to a single injection of 30 cobalt units sheep plasma erythropoietin in starved rats

Treatment[a]	h after injection	% Retics (Means ± standard errors)	Young, grade I retics positive response/ total No. of rats
Erythropoietin (6)	6	0.78 ± 0.06	0/6
Control (6)	6	0.50 ± 0.07	0/6
Erythropoietin (6)	12	0.60 ± 0.11	2/6
Control (6)	12	0.38 ± 0.07	0/6
Erythropoietin (6)	24	0.76 ± 0.08[b]	6/6
Control (6)	24	0.18 ± 0.02	0/6

[a] No. of animals in parentheses. Control group received equal volume of pyrogen-free saline. A total of 12 rats were used and each rat was sampled three times.
[b] $P < 0.01$.

Discussion. 12–24 h following a single injection of erythropoietin rats have higher reticulocyte percentages in their circulating blood than do control animals as well as significant numbers of reticulocytes bearing the stamp of morphologic immaturity. These effects were not observed in rats receiving either heat-inactivated erythropoietin extracts or saline injections. However, definitive proof of specificity of action awaits the availability of pure erythropoietin. A review of the literature reveals that the earliest reticulocyte changes reported are usually with 48 h[14–16] or 72 h[17] after an injection of erythropoietin.

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The present results lend support to the hypothesis that erythropoietin has some action upon erythrocytic precursors in the bone marrow that are advanced well beyond the stem cell stage as deduced by the rapid appearance of reticulocyte changes in the circulating blood. The large pool of rat bone marrow reticulocytes can be released into the circulating under certain conditions[18], and erythropoietin may play a role in such phenomena. Thus, erythropoietin will cause a release of isotopically labelled reticulocytes from the isolated perfused leg of a rat as early as 10 min after administration[19]. Finally, it is pertinent that in a series of parallel experiments performed at the same time and using Fe59 as a tracer, a significant increase in iron incorporation into circulating erythrocytes was demonstrated 12, 18, and 24 h following a single injection of erythropoietin[20]. Presumably, these increases are related to the reticulocyte changes that have been noted.

Zusammenfassung. Nach einer Injektion von Erythropoietin in hungernde Ratten beobachteten wir eine Erhöhung der Reticulocyten. Junge Formen von Reticulocyten erschienen bereits 12 h nach der Behandlung. Dieser Befund deutet vielleicht darauf hin, dass Erythropoietin nicht nur die Stammzellen angreift.

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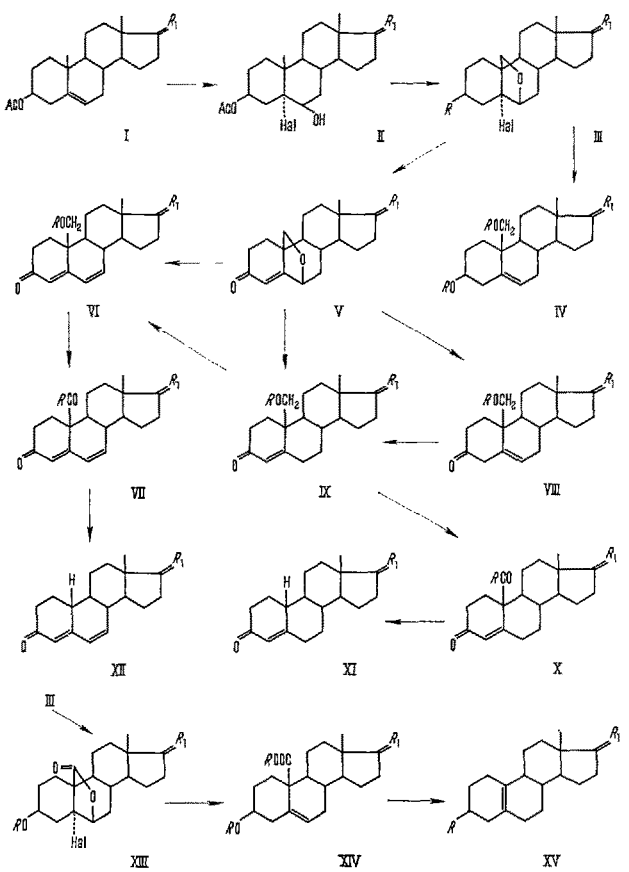
Ein neues Verfahren zur Herstellung von 19-Norsteroiden[1]

Für die Gewinnung von 19-Norsteroiden wird bis heute fast ausschliesslich die Reduktion von Verbindungen mit aromatischem Ring A (insbesondere von Oestronderivaten)[2a,b] nach BIRCH mit einem Alkalimetall in flüssigem Ammoniak verwendet. In letzter Zeit sind nun eine Reihe von Reaktionen bekannt geworden, die eine Oxigenierung der angulären Methylgruppe C-19 bewirken und damit eine direkte Eliminierung dieser Gruppe ohne Aromatisierung des Rings A möglich erscheinen lassen[3].



Wir berichten im folgenden über eine in den letzten zwei Jahren[4] in unsern Laboratorien bearbeitete, allgemein anwendbare und einfache Methode zur Gewinnung von 19-Norsteroiden. Durch Anlagerung unterchloriger[5] oder unterbromiger[6] Säure an Δ^5-3β-Acetoxy-steroide I erhält man die Halogenhydrine II. Wie die in 5-Stellung unsubstituierten, sekundären 6β-Hydroxy-steroide[7,8] lassen sich auch die Halogenhydrine II[9] in die 6β,19-Äther III umwandeln. Wir benützten dazu sowohl die Hypojoditreaktion[8,10] als auch die Reaktion mit Bleitetraacetat[7] (in Cyclohexan)[11], wobei im ersten Falle (insbesondere bei Verwendung von Bleitetraacetat und Jod in siedendem Cyclohexan) Ausbeuten bis zu 85% erhalten werden. Die 5α-Halogen-6β,19-Äther sind ausserordentlich stabil: mit Methylmagnesiumjodid kann z. B eine 17-Oxogruppe in eine 17α-Methyl-17β-hydroxy-Gruppierung umgewandelt und eine 20-Oxogruppe kann in bekannter Weise ins $\Delta^{17(20)}$-Enolacetat übergeführt werden, was die

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