1/4 CuCN + 3/4 $Na^{14}CN$ durch Sandmeyer-Reaktion in *o*-Nitrobenzonitril-(^{14}CN) übergeführt. Durch Verseifen der Nitrilgruppe und Reduktion der Nitrogruppe erhält man Anthranilsäure-($^{14}COOH$). Die spezifische Aktivität unseres Präparates betrug $1{,}06 \cdot 10^9$ Ipm/mMol.

21,5 mg Anthranilsäure-($^{14}COOH$) wurden an 10 drei Wochen alte bewurzelte Blätter über die Wurzel verfüttert. Nach 10 Tagen wurde der Versuch abgebrochen und das Pflanzenmaterial getrocknet. Trockengewicht der Wurzeln 1,37 g und der Blätter 3,44 g. Die Wurzeln wurden nach Alkalisieren 3 h mit Chloroform:Isopropanol (3:1) am Rückfluss erhitzt. Der Extrakt wurde eingeengt, mehrfach mit 5%iger HCl ausgeschüttelt und das Alkaloid nach Alkalisieren (pH 9) mit CH_2Cl_2 extrahiert. Chromatographisch konnte nur Peganin nachgewiesen werden. Die quantitative Bestimmung nach GRÖGER und JOHNE[6] ergab 16,6 mg Peganin. Zur eingeengten Methylenchloridlösung wurden 95 mg inaktives Peganin hinzugegeben, und das Ganze wurde an einer Al_2O_3-Säule basisch «Woelm» chromatographiert. Eluiert wurde mit Chloroform:Methanol (9:1). Die Peganin enthaltenden Fraktionen wurden zur Trockne eingeengt und einer Hochvakuumsublimation (10^{-2} Torr, 135°C) unterworfen. Die erhaltenen 89 mg Peganin wurden nochmals mit 123 mg inaktivem Peganin verdünnt. Zum Abbau wurden 210 mg (II), welches eine konstante Radioaktivität zeigte, eingesetzt. Die Oxidation wurde in Anlehnung an SPÄTH und NIKAWITZ[4] durchgeführt.

Das Alkaloid wurde in 150 ml Aceton p.a. gelöst. Zu dieser Lösung wurden in 4 Portionen insgesamt 430 mg $KMnO_4$ hinzugefügt, das Ganze wurde 4 h geschüttelt. Die entstandene 4-Oxo-3,4-dihydro-chinazolyl-3-essigsäure (III) haben wir extrahiert und nach Vertreiben des Lösungsmittels mit Diazomethan verestert (= (IV)). Das Rohprodukt wurde mit 20%iger KOH 40 min bei 95°C erhitzt. Nach dem Erkalten haben wir mit HCl neutralisiert und das Ganze mit Pufferlösung auf pH 3,5 eingestellt. Es wurde erschöpfend mit Äther extrahiert, danach das Lösungsmittel stark eingeengt und die Anthranilsäure auf DC-Platten gereinigt (Kieselgel G «Merck», angerührt mit 0,5%iger KOH; Lösungsmittelsystem Chloroform:Methanol:Eisessig (85:13:2). Vom Adsorbens liess sich (I) mit einer Mischung von Methanol:Aceton:Eisessig (8:2:1) eluieren. Das Eluat wurde zur Trockne gebracht, der Rückstand mit Pufferlösung pH 3,5 aufgenommen und mit Äther ausgeschüttelt. Der Ätherrückstand ergab 45 mg Anthranilsäure, die mehrfach im Hochvakuum sublimiert wurde. Smp. 144°C. Der Mischschmelzpunkt zeigte keine Depression. Das IR-Spektrum stimmte völlig mit einer authentischen Probe überein. Alle Radioaktivitätsmessungen erfolgten in unendlich dünner Schicht mittels des Methandurchflusszählers der Firma Friesecke und Höpfner.

Spezifische Radioaktivität des eingesetzten Peganins und der durch Abbau erhaltenen Anthranilsäure

Substanz	Spez. Aktivität Ipm/mMol	% der Gesamtaktivität
Peganin	$1{,}79 \cdot 10^6$	100
Anthranilsäure	$1{,}78 \cdot 10^6$	99,2

Summary. Anthranilic acid-($^{14}COOH$) was administered to rooted leaves of *Adhatoda vasica* Nees. The isolated alkaloid peganine = vasicine was degraded according to the method of SPÄTH and NIKAWITZ (Figure). The anthranilic acid obtained showed the same specific radioactivity as the alkaloid. Therefore anthranilic acid must be regarded as a direct precursor of peganine in *A. vasica* Nees.

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Pathway of Glucose Oxidation in Leucocytes Incubated in Different Conditions

According to BECK[1] the respiration of polymorphonuclear leucocytes incubated in phosphate medium is mainly related to the phosphogluconate oxidation pathway by means of a $NADP^+$-NADPH shuttle between it and the cytochrome chain. Experimental work carried out in this laboratory demonstrated that the respiration of resting polymorphonuclear leucocytes is very low and sensitive to amytal, antimycin and cyanide when the cells are incubated in phosphate medium and CO_2 is absorbed in KOH; it is dramatically increased, and turns out to be inhibited by amytal, but antimycin- and cyanide-insensitive, when the incubation is performed in Ringer bicarbonate containing glucose[2,3]. Since both bicarbonate and glucose are needed for this oxidative switch to be produced, the Crabtree effect, which is clearly shown in phosphate solution, no longer appears in the presence of bicarbonate[2].

This finding led to the hypothesis that in the presence of bicarbonate-CO_2 the carboxylation of pyruvate to malate, in cooperation with NADPH, would be allowed by the continuous supply of pyruvate through glycolysis not controlled by an efficient oxidative phosphorylation[2]. If this is so, an increased activity of the HMP pathway must be expected in the presence of bicarbonate, through regeneration of $NADP^+$. Since malic-dehydrogenase would ultimately transfer electrons to NAD^+, such carboxylative transhydrogenation could explain the oxidative switch exhibited by leucocytes in the presence of glucose and bicarbonate.

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Table I. Oxygen uptake and $C^{14}O_2$ production from glucose-1-C^{14} and glucose-6-C^{14} of resting polymorphonuclear leucocytes incubated in Ringer-phosphate, Ringer-phosphate with serum and Ringer bicarbonate solutions. About 30 million cells were incubated for 40 min at 38°C in 2.5 ml. The results are given as means ± S.D. with the number of experiments in each group shown in parentheses

	Krebs-Ringer phosphate	Krebs-Ringer phosphate with serum	Krebs-Ringer bicarbonate
Oxygen consumed μl	8.16 ± 0.760 (17)	16.40 ± 1.480 (17)	28.65 ± 7.2 (17)
$C^{14}O_2$ from glucose-1-C^{14} * ($0.25\ \mu c$ in 7 μmoles)	19.913 ± 2.150 (17)	48.142 ± 5.500 (17)	46.280 ± 6.244 (17)
$C^{14}O_2$ from glucose-6-C^{14} * ($0.25\ \mu c$ in 7 μmoles)	1.278 ± 195 (8)	1.255 ± 82 (8)	2.382 ± 286 (8)

* Total counts per 10 min per 30 million cells.

In this communication, experiments carried out measuring the $C^{14}O_2$ production from glucose-1-C^{14} and glucose-6-C^{14} in phosphate and bicarbonate solutions are presented. Since the addition of serum is also known strongly to stimulate the oxygen uptake of resting polymorphonuclear leucocytes[4,5], the experiments were extended to this condition.

In these experiments, the effect of amytal on the oxygen uptake and on the decarboxylation of glucose through the phosphogluconate oxidation pathway showed relevant differences between leucocytes incubated in the different conditions employed.

Methods. Guinea-pig polymorphonuclear leucocytes obtained by sterile peritoneal exudate (following injection of 1% NaCl) were used[6]. The cells were sedimented at low speed (400 *g*) for 10 min and resuspended in Krebs Ringer phosphate, Krebs Ringer phosphate with 20% fresh guinea-pig serum and Krebs Ringer bicarbonate solutions. The solutions contained 2.9 μmoles per ml of unlabelled glucose, taking into account that contained in serum.

In the experiments in which phosphate buffer was used, the oxygen uptake was measured with the Warburg direct method.

In the experiments in which leucocytes were incubated in bicarbonate buffer, the respiration was measured with the Warburg indirect method and calculations were made with the corrections for CO_2 retention (by leucocyte suspension and by amytal). In the experiments with labelled substrates, $C^{14}O_2$ was absorbed on KOH, precipitated as $BaC^{14}O_3$ and counted at infinite thickness[7,8].

Labelled substrates were supplied from the Radiochemical Dentre, Amersham (England); Amytal (sodium salt; pH checked and adjusted before using) from Lilly, Indianapolis (USA).

Results and discussion. The data presented in Table I demonstrate an increased activity of the hexose monophosphate pathway for glucose oxidation, together with an increase in respiration, obtained in the presence of serum or bicarbonate. The CO_2 production from glucose-6-C^{14} also appears to be increased by bicarbonate.

The effects of amytal on the yield of $C^{14}O_2$ from glucose-1-C^{14} and acetate-1-C^{14} (Table II) indicate that, while for the latter a complete inhibition is shown, the decarboxylation of C-1 of glucose is only partially sensitive to the inhibitor in phosphate medium, and that its increased activity (Table I) in serum-phosphate or bicarbonate solutions is produced through quite different mechanisms of reoxidation of NADPH. These differences are less pronounced but clearly shown for the oxygen uptake in the conditions employed.

It must be mentioned that the anaerobic production of $C^{14}O_2$ from glucose-1-C^{14} is in any case a small fraction of that produced under aerobic conditions[9].

The fact that both the oxygen uptake and the activity of the hexose monophosphate pathway of leucocytes incubated in phosphate medium are low, and only in part dependent upon an amytal-sensitive electron transport system, indicates that the reoxidation of the reduced coenzymes can diverge through two different paths, one being amytal-inhibited and clearly stimulated by the presence of bicarbonate, the other amytal-insensitive and stimulated by the addition of serum. The effect of serum is similar to that found in larger proportion during phagocytosis, when an amytal-insensitive stimulation of the oxygen uptake and of the phosphogluconate oxidation pathway takes place[7,10] and NADPH can be directly reoxidized by granules whose oxidase activity is greatly enhanced[7,11]. The effect of bicarbonate could be explained by the above-mentioned hypothesis[2,3] which is consistent with the present results. $C^{14}O_2$ fixation has been directly

Table II. Percentage inhibition by $2 \cdot 10^{-3}$ *M* Amytal on the oxygen uptake and $C^{14}O_2$ production from glucose-1-C^{14} and acetate-1-C^{14} of resting polymorphonuclear leucocytes incubated in Ringer-phosphate, Ringer-phosphate with serum and Ringer bicarbonate solutions. The values are given as mean ± S.D. from 8 experiments for each group

	Krebs-Ringer phosphate	Krebs-Ringer phosphate with serum	Krebs-Ringer bicarbonate
Oxygen uptake	62.8 ± 15	45.3 ± 11	76.0 ± 9
$C^{14}O_2$ from glucose-1-C^{14}	28.9 ± 11	07.0 ± 1.5	56.5 ± 12
$C^{14}O_2$ from acetate-1-C^{14}	89.6 ± 5	88.9 ± 6.5	86.5 ± 4

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demonstrated for leucocytes in lactate, and here exclusively in the carboxyl group[12,13] which corresponds to a fixation of $C^{14}O_2$ via a $C_1 + C_3$ condensation followed by equilibration of the activity of the carboxyl carbons via a symmetrical dicarboxylic acid. Therefore, and in view of the results presented in this communication, the effects of bicarbonate in increasing the activity of the hexose monophosphate pathway would suggest that pyruvate is carboxylated to malate in cooperation with NADPH. Malic dehydrogenase would then substitute NADH for NADPH and the increase of oxygen uptake produced by bicarbonate would be related to reoxidation of NADH through an amytal-sensitive pathway. The relevance of this pathway is demonstrated by the extent of inhibition of the oxygen uptake and of the decarboxylation via the phosphogluconate oxidation pathway found in bicarbonate solution. This amytal-sensitive, i.e. probably flavoprotein, step is not followed by an antimycin- and cyanide-inhibited respiratory chain[2,3].

Recently, WARBURG and his associates claimed that the aerobic glycolysis of leucocytes and other normal tissues should be considered as an artifact due to unsuitable handling, in contrast to the true aerobic glycolysis which would be peculiar to tumor cells[14].

As previously shown in this laboratory[2] and confirmed by others[15], when the procedure followed by WARBURG to avoid damage to the white blood cells is used, the leucocytes obtained are mostly lymphocytes, and aerobic glycolysis cannot be demonstrated with these cells, whilst it is characteristic for polymorphonuclear leucocytes.

With respect to the present experiments, this is a point of considerable interest, since the strong effect of bicarbonate on the aerobic metabolism, as far as the reductive carboxylation of pyruvate is concerned, cannot be produced except in the presence of an active aerobic glycolysis[2].

Riassunto. L'entità del consumo d'ossigeno e della produzione di $C^{14}O_2$ da glucosio-1-C^{14} e glucosio-6-C^{14} e la loro differente sensibilità all'amital nei leucociti polinucleati di essudato incubati in diverse condizioni dimostrano l'esistenza di più sistemi di riossidazione dei coenzimi ridotti.

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The Distribution of some Enzymes Involved in the Steroidogenesis of Hen's Ovary

Evidence for the secretion of sex hormones by the avian ovary has been given by LAYNE et al.[1,2]. Progesterone and oestrogens (oestrone, oestradiol-17β, oestriol) have been identified in the ovarian extracts of the laying hen by chromatographic mobility, UV-spectrophotometry and chemical reactions.

Efforts to identify the cell types in which steroidogenesis takes place have not so far been successful. Only recently Δ^5-3β-hydroxysteroid dehydrogenase (Δ^5-3β-HSDH) has been demonstrated histochemically in the granulosa and the theca interna of the growing oöcytes, atretic follicles and post-ovulation follicles of laying hens (BOTTE[3]). The presence of this enzyme merely indicates the existence of one of the first steps of steroid biosynthesis. In fact, it is demonstrable in all types of steroid-producing cells (adrenal, ovary, testis and placenta) and is involved with Δ^5-3-ketoisomerase in the transformation of pregnenolone to progesterone (see WETTSTEIN[4]). Recently AOSHIMA et al.[5], using a biochemical method, have even demonstrated Δ^5-3β-HSDH in the liver and kidney of the rat.

In this communication, we report the distribution of two enzymes of oestrogen metabolism in the ovary of laying hens (common hen of Aversa): DPN- and TPN-dependent 17β-hydroxysteroid dehydrogenase (17β-HSDH). These enzymes catalyse dehydrogenation of 17β-hydroxysteroids to 17-ketosteroids, and consequently oestradiol-17β to oestrone. They have so far been demonstrated biochemically in the human placenta (LANGER and ENGEL[6], TALALAY et al.[7], HOLLANDER et al.[8], HAGERMAN and VILLEE[9]), and in the liver of amphibia and fishes (BREUER et al.[10]), and histochemically in the human placenta (KELLOG and GLENNER[11], KLEINER et al.[12]) and in the rat liver and intestine (PEARSON and GROSE[13]).

The distribution of both 17β-HSDHs has been compared to that of Δ^5-3β-HSDH on contiguous sections. The histochemical reaction of WATTENBERG[14], modified by LEVY et al.[15], has been used to demonstrate Δ^5-3β-HSDH. DPN- and TPN-dependent 17β-HSDHs have

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