Ergebnisse und Diskussion. Kalli entstanden nur an Explantaten auf dem Medium 1a (Tabelle). Bei der anschliessenden Kultivation des Kallusgewebes auf diesem Nährmedium waren die Zellwände zunächst überwiegend braun gefärbt und stark strukturiert. Erst nach 3 Monaten entstand ohne Veränderung der Kulturbedingungen hellgelber, lockerer, wüchsiger Kallus. Er wurde auch auf die Nährmedien 1b und 2 (Tabelle) übertragen. Dieses Material verzehnfacht sein Frischgewicht während einer Subkultur. Es bildet Alkaloide, die teilweise in das Medium abgegeben werden. Wie bei der als Ausgangsmaterial verwendeten *M. microcarpa* treten vor allem Protopin und Allokryptopin auf. Auch das gegenseitige Verhältnis dieser Alkaloide entspricht im wesentlichen dem in der intakten Pflanze. Sanguinarin ist in der Gewebekultur unregelmässig vorhanden. Während einer Subkultur produzieren die Zellen 0,20 bis 0,25% ihrer Trockensubstanz an Protopin und Allokryptopin. Als Analysenwert für die gesamte *M. microcarpa*-Pflanze fanden wir 0,32% der Trockensubstanz an Protopin und Allokryptopin (Blätter 0,17%, Stengel 0,16%, Wurzel 1,6%). Die Biosyntheseleistung der Gewebekultur ist also vergleichbar mit der Produktivität der Gesamtpflanze.

Die von NEUMANN und MÜLLER[3] beschriebenen gelben Zellen können lichtmikroskopisch nachgewiesen werden. Sie ergeben bei Behandlung der Gewebeschnitte mit Mandel-Reagenz einen Niederschlag, der auf eine Anhäufung von Alkaloiden schliessen lässt. Ihre Färbung wird jedoch nicht, wie früher angenommen[3], durch Sanguinarin allein verursacht, denn auch Sanguinarin-freie Gewebekulturen enthalten gelbe Zellen. Der verantwortliche gelbe Farbstoff besitzt nach seinem Verhalten bei der Isolierung von Alkaloiden aus der Gewebekultur keinen Alkaloidcharakter. Trotzdem weist sein Vorkommen auf die Bildung und Speicherung von Alkaloiden hin (Signalfaktor): Die Selektion und Kultivation von intensiv bzw. schwach gefärbten Teilen der *M. microcarpa*-Gewebekulturen führt in der Regel zu alkaloidreichen bzw. alkaloidarmen Gewebekultur-Linien, die beispielsweise 0,40% bzw. 0,10% Protopin und Allokryptopin bezogen auf die Trockensubstanz der Zellen besitzen. Linien mit verschiedenem Alkaloidgehalt unterscheiden sich nicht grundsätzlich in ihrer Wachstumsintensität.

Gewebekulturen aus *M. microcarpa* gehören damit nicht nur zu den wenigen Gewebekulturen von Alkaloidpflanzen, die Alkaloide in messbaren Mengen bilden, sondern gestatten im Zusammenhang mit dieser Fähigkeit kontinuierliche Untersuchungen über Differenzierungsvorgänge. Sie weisen zugleich auf die Bedeutung von Signalfaktoren für die Selektion[8] auch bei einem von der intakten Pflanze abgeleiteten System hin.

Summary. Tissue cultures of the Papaveracee *Macleaya microcarpa* retained the capability of synthesizing alkaloids (protopine, allocryptopine, sanguinarine) for 3 years after initiation. The alkaloids are accumulated in single, yellow-coloured cells scattered in the tissue. The colour of these cells is continuously produced by a yellow pigment without alkaloidal properties. The orange alkaloid sanguinarine occurs only irregularly. Nevertheless, selection of deep and weak coloured pieces of tissue cultures led to lines with a high and a low content of alkaloids, respectively.

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Glutathione Peroxidase Activity of Inorganic Selenium and Seleno-DL-Cysteine

In the course of evolution, biological systems have seized upon the relatively meager catalytic activity of certain metals, and have greatly amplified their activity by incorporation into a complex organic matrix. For example, inorganic iron has the capacity to decompose hydrogen peroxide with a catalytic coefficient of 10^{-5}. This 'catalase' activity is amplified 1000-fold by incorporation of the iron into a porphyrin ring, and is brought to its ultimate efficiency, an activity 10^8 to 10^{10} times as great as that of iron alone by the addition of the protein moiety of the enzyme, catalase[1].

It has recently been demonstrated that selenium forms an integral part of the glutathione peroxidase molecule, in rats[2], sheep[3], cattle[4], and man[5]. We now demonstrate that inorganic selenium also has well-defined glutathione peroxidase activity, albeit at a level considerably lower than that of the enzyme, glutathione peroxidase.

Sodium selenite was obtained from J.T. Baker, and seleno-DL-cystine and seleno-DL-methionine were purchased from Sigma Chemical Corporation. The hemolysate and all selenium compounds were dissolved in a solution containing 0.7 m*M* β-mercaptoethanol and 2.7 m*M* EDTA, pH 7.0. Presumably, the seleno-DL-cystine was reduced to seleno-DL-cysteine by the β-mercaptoethanol and GSH. Assays of glutathione peroxidase activity were carried out by linking the formation of oxidized glutathione (GSSG) to the oxidation of NADPH through glutathione reductase, according to the principle originally described by PAGLIA and VALENTINE[6]. The complete assay system for GSH-Px contained *Tris*-HCl, 0.1 *M*, pH 8.0 (25°); EDTA, 0.5 m*M*; GSH, 2.0 m*M*; glutathione reductase, 1 U/ml; NADPH, 0.2 m*M*; t-butyl hydroperoxide, 0.7 m*M*. The 'GSH-Px activity' of each preparation was determined by measuring at 37 °C the decrease in optical density at 340 nm of the complete system against a blank from which only the source of 'enzyme' (hemolysate, sodium selenite, seleno-DL-cysteine or other test substance) has been omitted. An additional

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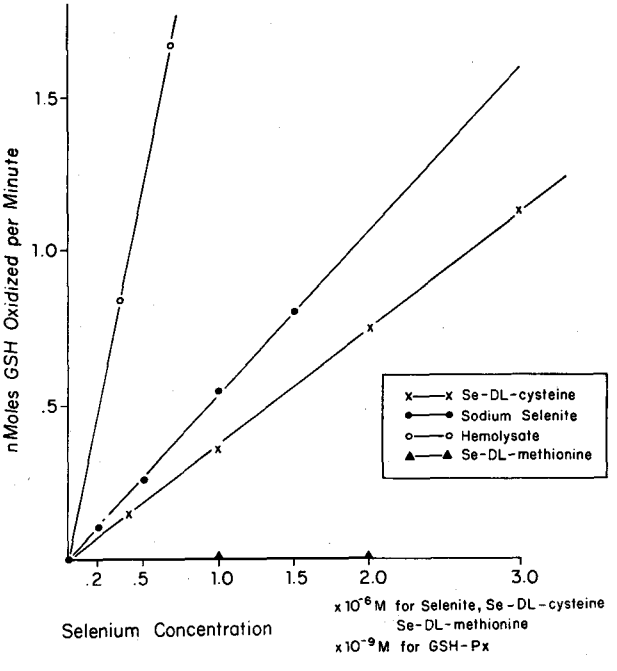
	Sodium selenite (*M*)	Seleno-DL-cysteine (*M*)	Hemolysate (*M*)
K_m GSH	9.9×10^{-4}	3.6×10^{-4}	1.02×10^{-3}
K_m t-butylhydroperoxide	2.3×10^{-4}	4.1×10^{-4}	3.2×10^{-4}
Energy of activation (Kcal)	8.78	8.30	2.85

blank determination was also carried out at each substrate concentration: the change of optical density was measured in a system containing 'enzyme' but lacking t-butyl hydroperoxide against one lacking both 'enzyme' and t-butyl hydroperoxide. This blank corrected for non-enzymatic oxidation of glutathione by the 'enzyme'. The Figure shows the glutathione peroxidase activity of various concentrations of hemolysate, sodium selenite, seleno-DL-cysteine and seleno-DL-methionine. The GSH-Px selenium content was estimated from the hemolysate enzyme activity based upon the fact that homogeneous GSH-Px from human red cells has a specific activity of 100 U/mg protein[5]. Sodium selenite and seleno-DL-cysteine were both found to have marked GSH-Px activity at concentrations well under 1 μM/l. Seleno-DL-methionine, on the other hand, lacked GSH-Px activity. Although sodium selenite and seleno-DL-cysteine had well-marked GSH-Px activity, and this activity was linear with respect to selenium concentration, the activity of hemolysate GSH-Px was much higher, approximating about 5,000 times the activity of sodium selenite and seleno-DL-cysteine when calculated on the basis of selenium content.



The rate of oxidation of glutathione (GSH) by selenium (Se-DL-cysteine, sodium selenite, Se-DL-methionine, and GSH-Px [hemolysate]) as a function of concentration of selenium. In the case of the hemolysate, selenium concentration has been estimated on the basis of the known selenium content of red cell glutathione peroxidase (GSH-Px)[5].

Kinetic studies of sodium selenite, seleno-DL-cysteine, and hemolysate GSH-Px were also carried out. When the concentration of either of the substrates, t-butyl hydroperoxide or GSH, was varied, the rate of oxidation of GSH was found to follow Michaelis-Menton kinetics. The K_m for t-butyl hydroperoxide was determined with a GSH concentration of 2 mM and that for GSH, with a t-butyl hydroperoxide concentration of 0.7 mM. The energy of activation was calculated from Arrhenius plots at temperatures ranging from 35 to 45 °C, in the case of hemolysate, and 25 to 45 °C in the case of seleno-DL-cysteine and seleno-DL-methionine. The results of our studies are summarized in the Table.

The sodium salts of the following substances were tested for GSH-Px activity at 1 μM concentration: sulfite, sulfate, tellurite, tellurate, telluride and selenate. None had detectable GSH-Px activity ($< 10\%$ of the activity of selenite).

It has been known for many years that sodium selenite catalyzes the autooxidation of GSH. It has been suggested that a selenite GSH complex, of the form G-S-Se-S-G is an intermediate in this process and decomposes to GSSG and Se[7,8]. We suggest that the glutathione peroxidatic effect of selenite is exerted through coupling of the oxidation of GSH by selenite to the reoxidation of the elemental selenium formed to selenite by t-butyl hydroperoxide. The putative reaction sequence may be summarized as follows:

$$4\text{GSH} + \text{H}_2\text{SeO}_3 \rightarrow \text{GS–Se–SG} + \text{GSSG} + 3\text{H}_2\text{O}$$
$$\text{GS–Se–SG} \rightarrow \text{GSSG} + \text{Se}$$
$$\text{Se} + 2\text{R–O–OH} + \text{H}_2\text{O} \rightarrow \text{H}_2\text{SeO}_3 + 2\text{R–OH}$$

where R is the t-butyl group.

The affinity of selenite or seleno-DL-cysteine for the two substrates, GSH and t-butyl hydroperoxide is similar to that of the complete enzyme. The main difference between the enzymatic form of selenium and inorganic or amino acid selenium is in the energy of activation. It is of interest that seleno-DL-methionine, in which the selenium atom is bonded to two carbons, is ineffective as a GSH-Px.

The glutathione peroxidase activity of selenium represents another, new, example of enhancement of the catalytic activity of a metal by its incorporation into a protein.

Zusammenfassung. Glutathionperoxidase von Säugetieren ist als Selen enthaltendes Enzym bekannt. Die durch das Enzym gesteuerte Reaktion wird auch durch Natriumselenit und Seleno-Cystin katalysiert, wobei die Aktivität dieser Verbindungen jedoch nur 1/5000 derjenigen des Selens im menschlichen Erythrozytenenzym entspricht.

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