followed by the estimation of formic acid liberated and uptake of periodate showed the presence of only one glucose moiety in the glucoside.

Methylation of glucoside followed by the acid hydrolysis resulted in the isolation of a compound m.p. 176–78°, it was recrystallized from methanol as fiery red meedles, m.p. 177–78°. The compound was identified to be β-methyl physcion[14], m.p. 178°.

Isolation of β-methyl physcion from the hydrolysis of methylated glucoside indicates that free hydroxyl group is at position 8 in the glucoside. This indicates that glucose moiety is attached at position 1. Hence glucoside has been identified as 1-glucoside of 1:8 dihydroxy-6-methoxy -3-methyl anthraquinone or 1-glucoside of physcion.

Zusammenfassung. In *Cassia occidentalis* Linn. wurde das 1-Glukosid des 3-Methyl-6-methoxy-1,8-dihydroxy-anthrachinons nachgewiesen.

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Structural Characterization of Immunoglobulins Contained in Polyacrylamide Gels

Polyacrylamide gel electrophoresis is widely used in biochemistry due to its good resolution. However, doubts often exist about the precise identitity of separated proteins. For some, specific enzymatic reactions[1,2] or satining methods[1–3] are available. Associations of polyacrylamide gel electrophoresis with immunodiffusion using specific antisera have been frequently used to identify proteins separated from a mixture[1,3–10].

As an alternative for the identification of immunoglobulins, a method will be described where the protein, contained in polyacrylamide gel slices, is reduced in the presence of sodium dodecyl sulphate (SDS), followed by electrophoretic determination of the molecular weight of the sub-units released to the supernatant.

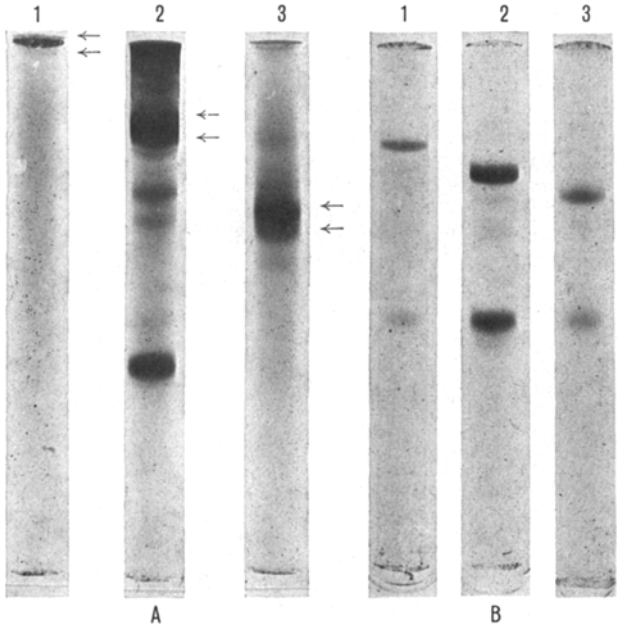

Fig. 1. Structural characterization of immunoglobulins contained in polyacrylamide gels. The 3-gel set A illustrates sodium dodecyl sulphate-polyacrylamide gel separation of 25 μg of a polymeric monoclonal IgM protein (gel 1), 75 μg of a partially purified dimeric monoclonal IgA protein (gel 2), and 50 μg of isolated polyclonal IgG (gel 3). The arrows indicate the regions sliced in twin, frozen gels, for reduction and alkylation. 2 to 3 slices of each gel were transferred to small test tubes, and 50 to 100 μl of sodium dodecyl sulphate, dithioerythritol – contaning buffer added to each tube. After reduction was completed, a supernatant was collected and run in the 3-gel set B. Gel 1 of this set correspond to reduction of IgM, gel 2 to reduction of IgA, and gel 3 to reduction of IgG. (For details, see text.)

Material and methods. Proteins. Immunoglobulins G, A, and M were isolated as described previously[11,12]. Chymotrypsinogen A and ovalbumin were purchased from Serva, human albumin from Fluka, and lactate dehydrogenase and catalase from Sigma.

Acrylamide electrophoresis. Electrophoretic separations were realized in 70×6 mm polyacrylamide gel discs, containing 0.1% (w/v) sodium dodecyl sulphate, according to SUMMERS, MAIZEL and DARNELL[13]. The initial separation of a given immunoglobulin was made on a 4.25% (monomer w/v) gel, and mol. wt. calculation was based on data collected from runs on 7.5% (monomer w/v) gel.

Reduction of proteins contained in acrylamide gel. Samples were run in duplicate, one gel cylinder to be frozen on dry ice, the other to be stained[10]. The position of the protein to reduce on the frozen gel was calculated from its mobility on the stained gel, after convenient correction for shrinking in the stain solution and individual differences among gels separated in the same run. The frozen gel was transversally sliced in 1 mm width sections, and 2 to 3 slices calculated to contain a given immunoglobulin were placed in a small test tube, to which 50 to 100 μl of 2% (w/v) sodium dodecyl sulphate in sodium phosphate buffer pH 7.2, 0.01 *M*, and 5 to 10 μl of 1 *M* dithioerythritol were added. Reduction was carried out at 100 °C for 2 periods of 10 min, separated by a 20 min period of reduction at room temperature. Proteins to be used as references were treated identically, except that

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Comparison of molecular weights of immunoglobulin heavy chains obtained by reduction of proteins in solution or contained in gel

Protein	Reference[19] (mol. wt.)	Protein in solution (Mol. wt.)	Protein in gel (Mol. wt.)
Human IgG	53,000	55,500	58,000
		56,000	57,000
		53,875	56,000
		m 55,125 (+ 4%)	m 57,000 (+ 7.6%)
Human IgA	64,000	64,000	66,000
		62,000	64,000
		m 62,000 (− 1.6%)	m 65,000 (+ 1.6%)
Human IgM	70,000	74,000	76,000
		72,000	75,000
		75,000	76,000
		m 73,833 (+ 5.5%)	m 75,500 (+ 7.9%)

Percents given in brackets refer to deviations from reference molecular weight values[19].

no previous separation in polyacrylamide gel was involved.

Molecular weight calculations from electrophoretic mobilities in the SDS – polyacrylamide gel system. Mol. wt. values for immunoglobulin heavy chains were extrapolated from a calibration curve constructed with the electrophoretic mobilities (calculated according to WEBER and OSBORN[14]) and molecular weights of reference proteins[15–17].

Results. In previous studies we demonstrated the possibility of obtaining mol. wt. estimations for human immunoglobulin heavy chains from their mobilities in sodium dodecyl sulphate-polyacrylamide gels within ±5% of the values reported in the literature[15, 16]. On the other hand, accurate mol. wt. determinations for complete immunoglobulin molecules are not possible, considering the large number of disulphide bonds and the complex tertiary structure, interfering with sodium dodecyl sulphate binding[18]. Mol. wt. values determined for the monomers of IgM and IgA and for IgG overlap, and identification on this basis is not trustworthy. A better way to identify an immunoglobulin separated by acrylamide gel electrophoresis appeared to be the determination of the molecular weight of its heavy chain. Simultaneous reduction of the protein contained in the gel slice and elution of free chains into the supernatant was found to be feasible, as illustrated by Figure 1. From the mobilities calculated for reference proteins and immunoglobulin heavy chains, it was posible to calculate the mol. wt. of the last, as shown in Figure 2. The Table allows the comparison between mol. wt. values for heavy chains obtained from proteins reduced in free solution or entrapped into acrylamide gel slices. Differences are observed – in general values obtained from proteins reduced within gel slices are 2.5 to 3.0% larger than those obtained from proteins in free solution – but the differences do not exceed 10% over reference values, and the obtained mol. wt. values allow the determination of the immunoglobulin class beyond any doubts.

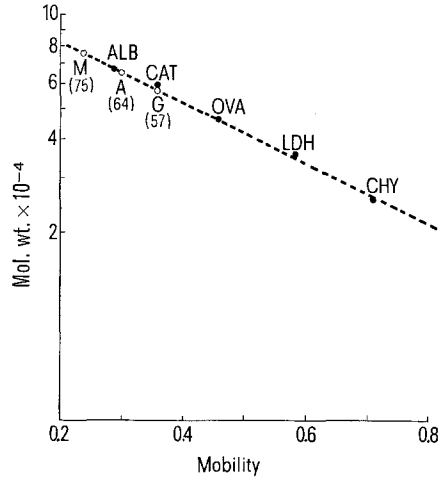

Fig. 2. Molecular weight calculation of heavy chains separated in sodium dodecyl sulphate – polyacrylamide gel electrophoresis. A plot of mobilities vs. log. molecular weight was built from the following control proteins: chymotrypsinogen A (CHy 25,700), lactate dehydrogenase (LDH, 36,000), ovalbumin (OVA, 46,000), catalase (CAT, 60,000) and serum albumin (ALB, 66,000). Mobilities for the heavy chains of IgG (G), IgA (A) and IgM (M), separated on the same run that control proteins, were plotted on the curve and the corresponding mol. wt. values calculated (calculated values × 10^{-3} are given by the bracketted figures in the plot).

Conclusions. The identification of immunoglobulins of the 3 major classes (IgG, IgA and IgM) can be based on the differences in mol. wt. of their heavy chains. This principle is applicable to immunoglobulins contained in acrylamide gels, since we have proved the possibility of reducing a protein contained in polyacrylamide gel slices, obtaining an eluate with free chains. Through this simple method the class of a given immunoglobulin can be determined from about 25 μg of protein contained in the gel.

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Résumé. L'identification des trois classes majeures d'immunoglobulines humaines peut avoir comme base la détermination des poids moléculaires de ses chaînes lourdes. Ce principe est applicable aux protéines contenues dans des gels de polyacrylamide, bien qu'on puisse les réduire directement à partir des sections de gel qui les renferment, en obtenant un éluat avec des chaînes libres qu'on peut étudier pour déterminer le poids moléculaire des chaînes lourdes.

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Erzeugung von Rigor der Rattenextremitäten durch Hemmung der Glyzin-Synthese

Es ist bekannt, dass kurzdauernde Unterbindung der Aorta (40 min) unter den Nierenarterien bei einigen Tieren zur Rigidität der Hinterextremitäten führt[1]. Wir konnten zeigen, dass bei Ratten diese Art von Rigidität durch Glyzin (G) verhindert werden kann[2]. Die Unterbindung der Aorta führt aber auch zum Verschwinden von G und zur Zerstörung der Interneuronen in der medulla spinalis, während die γ-Aminobuttersäure unverändert bleibt[3]. ARNSTEIN und STANKOVIĆ[4] zeigten, dass Mangel an Pteroyl-Glutaminsäure zur Hemmung der G-Synthese führt. Zur Prüfung, ob durch Hemmung der G-Synthese ein Rigor entstehen wird und ob dieser sich auch durch G aufheben lässt, haben wir ca. 150 g schweren, männlichen Wistar-Ratten 2 mg/kg/i.p. den Antagonist der Pteroyl-Glutaminsäure Aminopterin injiziert. 3–5 h später entwickelte sich der Rigor der Hinterextremitäten. Auf der höchsten Schwelle der Rigidität wurden 400 mg/kg/i.p. Glyzin injiziert, worauf der Rigor nach 30 min verschwand. Jede Gruppe bestand aus 10 Ratten und G wurde in jeweilen 2 medulla spinalis nach der Methode von BRENNER und NIEDERWIESER[5] bestimmt.

Glyzin in der Medulla spinalis von Ratten (γ/g)

		P	Bemerkung
1 Kontrolle	$5{,}95 \pm 0{,}19$		
2 Aminopterin	$4{,}13 \pm 0{,}13$	$< 0{,}01$	Signifikanz bezieht sich auf 1
3 Aminopterin + Glyzin	$5{,}08 \pm 0{,}11$	$< 0{,}01$	Signifikanz bezieht sich auf 2

Aus der Tabelle ist ersichtlich, dass es nach Aminopterin zu signifikanter Erniedrigung von G kam, während G nach der Injektion im Rückenmark erhöht war.

Aus diesen Resultaten kann man mit grosser Wahrscheinlichkeit schliessen, dass der durch kurzdauernde Unterbindung der Aorta hervorgerufene Rigor durch das Verschwinden der Interneuronen und die Hemmung der Glycinsynthese in der medulla spinalis entsteht. Zugleich kann dadurch bewiesen werden, dass Glyzin die hemoencephale Barriere durchdringt, was wir schon früher an Mäusen zeigen konnten[6]. Es stellt sich die Frage der eventuellen Glyzin-Anwendung bei gestörter Durchblutung des Rückenmarks in der Humanpathologie.

Summary. Inhibition of glycine synthesis by aminopterine, an antagonist of pteroilglutaminic acid, causes rigidity of hind limbs in rats. This rigidity can be abolished by the injection of glycine.

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The Incorporation of ^{45}Ca into the Egg of the Medaka, *Oryzias latipes*

Ca may play an important role in the fertilization process of animals[1–3]. It might, therefore, be interesting to trace inter- and intra- cellular movements of calcium upon the fertilization. RUDENBERG[4], HSIAO and BOROUGHS[5] tried to measure the radioactivity of ^{45}Ca which penetrated into the egg of sea urchin by in vitro incubation. It seemed likely that in those experiments the radioactivity of the penetrated ^{45}Ca was not enough to trace the movements of the element. However, there may be another possibility that the ovum accumulates the radioisotope from the body fluid during the period of the egg maturation and subsequently a high level of the intracellular concentration of ^{45}Ca can be expected. The purpose of the present experiment is, therefore, to deal with the incorporation of radioactive calcium into the egg of the medaka which is injected intra-abdominally.

The egg of the medaka, *Oryzias latipes* (orange-red variety) was used as material. Ringer's solution which

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