Complement fixation titers

Antigen	Serum from rabbits immunized with crude antigens from types				Serum from rabbits immunized with purified antigens from types			
	3	4	5	7	3	4	5	7
Type 5 crude antigen	> 128	> 128	> 128	> 128	16	4	128	16
Type 5 purified antigen	32	64	32	32	4	2	64	4
Type 3 crude antigen	n. t.*				16	32	32	16
Type 3 purified antigen	n. t.				16	4	32	8
Type 7 crude antigen	n. t.				8	8	32	8
Type 7 purified antigen	n. t.				8	4	32	8

* n.t. = not tested.

Untreated tissue culture fluids of these viruses (crude antigens) and fluorocarbon treated cultures (purified antigens) were used respectively. The four types of adenoviruses had been grown in cultures of HeLa cells until complete cellular degeneration occurred. The crude antigens consisted of the whole culture fluids and were used to immunize rabbits by means of five semi-weekly intravenous injections of 1·5 ml each, the rabbits being bled one week after the last injection[3]. For the preparation of the purified antigens, the culture fluids and cellular debris, first homogenized in a TenBroeck grinder, were mixed with an equal volume of 1,2-difluorotetrachlorethane (Freon 112, Du Pont de Nemours), which had been dissolved in *n*-heptane until a specific gravity of 1·30 was obtained. The mixtures were blended at top speed in a Servall Omni-Mixer during 3 min in the cold. The homogenates were then centrifuged at 1,500 r.p.m. for 15 min. The clear aqueous upper layers, containing the viruses, were furthermore centrifuged at 26,000 r.p.m. for 60 min in head No. 40 of the Spinco centrifuge. The supernatant fluids containing most of the group-specific complement fixing 'soluble' antigen[4] were discarded and the sediments, containing the viral antigens, were resuspended in a volume of Hanks' balanced salt solution sufficient to achieve a ten-fold concentration of the original culture. These suspensions were used for immunizing rabbits by means of five semi-weekly intravenous injections of 0·25 ml each. The rabbits were bled one week after the last injections. For the complement fixation tests, the antigens were prepared from types 3, 5 and 7 adenoviruses. The viruses were grown in cultures of HeLa cells; after complete cellular degeneration, the whole cultures were homogenized in a TenBroeck grinder. One half of each culture was used as such (crude antigens). The other half was treated with fluorocarbon according to the procedure described above (purified antigens). The virus containing upper layers were not centrifuged in the Spinco centrifuge, but used as such.

All sera and antigens were inactivated at 56°C for 30 min. Neither kind of antigen had any appreciable anticomplementary activity. Two full units of complement were added to each antigen-serum dilution mixture and the hemolytic system was added after incubation for 30 min at 37°C and storage overnight in the refrigerator. 50% hemolysis was taken as the end point. The CF titers obtained are indicated in the Table.

From the example of type 5 adenovirus, it can be seen that the fluorocarbon purification of the immunizing virus did effectively prevent the appearance in the serum of the high titer, unspecific antibodies obtained when crude antigens are used for immunization. Furthermore, when sera from animals immunized with purified antigens are used in the complement fixation test, there is no significant difference in CF titers, whether the antigens used in the test have been previously purified or not: this fact indicates that, with these sera, reactions with host cell constituents are greatly minimized or abolished.

Type-specific complement fixation reactions were not observed however and there was considerable cross reaction between the four antigenic types, which may lend support to the already proposed hypothesis[5] that common antigens are shared by the viral particles of the different types. Further work is needed however to demonstrate a complete removal by this method of all nonviral antigenic substances.

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Wistar Institute University of Pennsylvania, Philadelphia, March 25, 1957.

Résumé

La purification préalable, par le fluorocarbone, d'adénovirus (types 3, 4, 5 et 7) servant à l'immunisation de lapins, prévient la formation dans le sérum de ces derniers d'anticorps fixateurs de complément, réagissant de manière non spécifique avec les constituants des cultures de cellules dans lesquelles les virus ont été propagés.

Cette méthode ne permet cependant pas d'obtenir des anticorps qui réagissent de manière spécifique de type avec les adénovirus, dans la réaction de déviation du complément.

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1-(3'-Methoxy-4'-hydroxy benzyl) 6-7 dimethoxy isoquinoline, a major metabolite of papaverine

Little is known about the metabolic fate of the opium alkaloid, papaverine, a potent coronary dilating agent and smooth muscle relaxant. After its administration to

a number of mammalian species, we have observed that considerable quantities of metabolites were excreted in the urine. These were extractable into ethylene dichloride, absorbed light in the ultraviolet region (max. 251, 312 mμ, min. 266 mμ) and reacted with several phenolic reagents[1]. There was a marked increase in the phenolic material when acidified urine was heated at 100°C for 1 h, indicating the presence of 'bound' phenolic products.

Phenolic material obtained from acid treated urine of guinea pigs that had received papaverine was extracted into ethylene dichloride at pH 7·0 and subjected to a 24 transfer countercurrent distribution with pH 3·9 citrate buffer and ethylene dichloride. The resulting distribution curve of the material absorbing at 251 mμ showed the presence of at least two compounds. The contents of plates 0–12, representing the major fraction of the total phenols, were pooled and again distributed in 24 plates with ethylene dichloride and pH 3·3 citrate buffer. Plates 10–16 showed the presence of a single phenolic product. These plates were pooled and the metabolite was extracted into ethylene dichloride at pH 7·0. After evaporation of the organic solvent the resulting compound was recrystallized twice from ethanol, yielding white crystals which melted at 162–164°C. An authentic sample of 1-(3′-methoxy-4′-hydroxy benzyl) 6-7 dimethoxy isoquinoline, (4′-hydroxy papaverine) melted at 162–164°C and a mixed melting point of the two compounds showed no depression. The ultraviolet absorption and a fluorescent spectra of the metabolite and 4′-hydroxy papaverine were identical. Additional evidence for the identity of 4′-hydroxy papaverine was obtained from its behavior on paper chromatograms and its partition coefficients between various buffers and ethylene dichloride. From the countercurrent distribution it was calculated that about 40 to 50% of the administered papaverine was excreted as 4′-hydroxy papaverine in the guinea pig. Similar results were obtained in human subjects.

Incubating papaverine with microsomes from guinea pig or rabbit liver, reduced triphosphopyridine nucleotide, and oxygen[2] resulted in cleavage of papaverine to yield formaldehyde and 4′-hydroxy papaverine, as well as other phenolic products.

After the oral administration of papaverine to human subjects, about 99% of the phenolic transformation products was excreted in the urine in the 'bound' form. Incubating the urine with mammalian β-glucuronidase resulted in the liberation of about 80% of the phenols, suggesting that they are conjugated with glucuronic acid. Previous studies have shown that cell-free preparations can synthesize glucosiduronic acids in the presence of uridine diphosphate glucuronic acid (UDPGA) and phenolic acceptors[3]. When 4′-hydroxy papaverine was incubated with guinea pig microsomes and UDPGA almost all of the drug disappeared. Treating the incubation mixture with β-glucuronidase resulted in a quantitative recovery of the free phenol. These observations were taken as evidence that the microsomal preparation can transfer glucuronic acid from UDPGA to 4′-hydroxy papaverine.

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Zusammenfassung

An Glukoronsäure gebundenes 1-(3′-Methoxy-4′-hydroxy-phenyl)-6, 7-dimethoxy-chinolin ist als Metabolit des Papaverins beim Menschen und bei Säugetieren erkannt und isoliert worden. Ein in den Lebermikrosomen enthaltenes Ferment, das sauerstoff- und TPNH-abhängig ist, bewirkt die Methoxylspaltung, und ein weiteres Ferment überträgt die Glukuronsäure aus ihrer Verbindung mit Uridinphosphat auf die entstandene phenolische Verbindung.

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Zur Gewinnung von Induktionsstoffen aus Hühnerembryonen

Bei der chemischen Aufarbeitung 7–9 Tage alter Hühnerembryonen wurden nach verschiedenen Methoden Proteinfraktionen voneinander getrennt, die nach der Implantation in Molchgastrulen in der präsumptiven Rumpfepidermis die Bildung von Vorderköpfen (*archencephale Induktion*[1]) oder von Rümpfen mit Schwänzen (*spinokaudale Induktion*[1]) hervorriefen[2]. Bei der Fortführung dieser Versuche konnten zudem Stoffe isoliert werden, welche in 80–100% der Fälle grosse Hinterköpfe (deuterencephal[1]) induzierten. Zum Teil liefen die Induktionen in einen Rumpf-Schwanz-Fortsatz aus. Zur Gewinnung dieser deuterenzephal-spinokaudal wirkenden Fraktionen wurden Hühnerembryonen homogenisiert und zentrifugiert. Der Rückstand wurde mehrmals mit 0,16 *m* NaCl-Lösung gewaschen, dann mit 0,05 *m* Pyrophosphatpuffer bei pH 9,5 bis 10,0 extrahiert und die wirksame Fraktion aus dem zentrifugierten Extrakt bei pH 6,5 gefällt. In der Tabelle ist als Beispiel Fraktion E 87 I + II aufgeführt. Diese Fraktion wurde bei pH 7,4 mit Thioglykolsäure inkubiert. Nach der Behandlung mit Thioglykolsäure wurden keine spinokaudalen Induktionen mehr hervorgerufen, während die deuterenzephale Induktionsfähigkeit nicht abgeschwächt war. Ausserdem induzierte die Fraktion jetzt archencephale Organe, welche vorher nicht gebildet worden waren.

Nach Hydrolyse mit Trypsin (0,1–1,0 mg/cm³, 60 min, 25°) nahm die Zahl der spinokaudalen und deuterencephalen Induktionen ab. Bei diesen Versuchen wurde die Fermentwirkung durch Zugabe von Sojabohnen-Trypsin-Inhibitor abgestoppt. Dadurch wurde verhindert, dass das Trypsin nach der Implantation in die Keime weiterwirkt. Ohne Abstoppen der Trypsinwirkung wurden die spinokaudale und die deuterenzephale Wirksamkeit bei den gleichen Fermentkonzentrationen sowie bei einer noch geringeren Konzentration (0,012 mg/cm³) vollständig aufgehoben.

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