und Chinin verringerten in Konzentrationen von $3{,}75 \times 10^{-6}$ bzw. 2×10^{-5} g/ml die Herzleistung (berechnet in erg/min) um 75–80%. Die Fluoressigsäure dagegen verminderte die Herzleistung trotz hoher Konzentrationen (2×10^{-4} g/ml) und starker Abnahme des Gehaltes an energiereichen Phosphaten nur wenig (ca. 33%). Nach Geninzugabe stieg zwar bei Fluoressigsäureschädigung die Kontraktionshöhe an, gleichzeitig verminderte sich jedoch die Herzfrequenz so hochgradig, dass die Herzleistung – die im wesentlichen das Produkt aus Hubhöhe und Frequenz darstellt – nicht ansteigt.

Hubhöhe und Frequenz der Vorhofpräparate wurden mit Torsionsmyographen photokymographisch registriert. Der cardiotone Effekt wurde aus der Differenz zwischen der Herzleistung vor Zugabe des Cardenolid (das heisst zur Zeit der maximalen Herzschädigung) und dem Maximum der positiv inotropen Wirkung, ausgedrückt in Prozenten und bezogen auf den Ausgangswert vor der Schädigung, berechnet.

Die Versuchsergebnisse sind in der Tabelle zusammengefasst.

Unabhängig von der Struktur der 6 Cardenolide und Bufadienolide führten äquieffektive Konzentrationen nach Herzschädigung mit 2,4-Dinitrophenol zu einer gleich starken Zunahme der Herzleistung. Nach Schädigung mit Fluoressigsäure dagegen sind die 3,14,12-Trioxyverbindungen stärker wirksam als die 3,14-Dioxy- ($P > 0{,}01$) und die 3,14,16-Trioxyverbindungen. Zwischen den einander entsprechenden 5- und 6-C-Laktonringderivaten war kein Unterschied nachweisbar. Nach Schädigung mit Chinin erhöhen bei den untersuchten Trioxyderivaten jeweils die Bufadienolide stärker die Herzleistung als die Cardenolide ($P < 0{,}01$). Ausserdem sind wieder die beiden 3,14,12-Trioxyderivate den 3,14,16-Trioxyverbindungen überlegen ($P < 0{,}02$ bzw. $P < 0{,}001$). Bei den 3,14-substituierten Verbindungen war zwischen 5- und 6-C-Laktonringderivat kein Unterschied nachweisbar ($P > 0{,}2$). Im Vergleich zu den 3,14,12-Trioxyverbindungen ist nur das Bufalin, nicht aber das Digitoxigenin den entsprechenden 3,14,12-Trioxyverbindungen unterlegen.

Mit der angegebenen Methode ist es möglich, an verschiedenen biochemisch und elektrophysiologisch definierten Herzschädigungsmodellen qualitative Unterschiede im Wirkungsmechanismus von Cardenoliden und Bufadienoliden in Abhängigkeit von Zahl und Stellung der OH-Gruppen und der Struktur des Laktonringes nachzuweisen.

Summary. By damaging isolated auricles of guinea pigs with 2,4-dinitrophenol, fluoroacetic acid or quinine, it was possible to obtain qualitative differences of effect depending upon the chemical structure of the cardenolids and bufadienolids.

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The Incorporation of 8-^{14}C-Adenine and 8-^{14}C-Guanine into PNA of *Rhodospirillum rubrum*

A number of investigations have been carried out to determine the ability of micro-organisms, which do not require preformed purines for growth, to incorporate ^{14}C-adenine and ^{14}C-guanine into the purine bases of PNA. The results have revealed that they can be divided into two main groups: a) those which incorporate guanine into PNA-adenine and guanine, and adenine exclusively or mainly into PNA-adenine, and b) those which incorporate guanine exclusively or mainly into guanine, and adenine into both PNA purines (see e.g. BROWN[1], GOODWIN[2]); an example of a) is *Mycococcus pyogenes* v. *aureus* and of b) *Aerobacter aerogenes*. The photosynthetic bacterium *Rhodospirillum rubrum* had not previously been examined in this respect, so as part of our studies on purine metabolism in this organism we undertook such an investigation.

R. rubrum was cultured anaerobically in the light under our standard conditions (GOODWIN and OSMAN[3]) and the 8-^{14}C-purine added to 48 h cultures. The cells were harvested 6 h later, washed repeatedly with 80–90% ethanol, ethanol-ether (1:1) and finally ether, and extracted twice with 10% aqueous NaCl (7 ml/1 g dry weight cells) under reflux at 100° for 1 h. The pooled extract was cooled to 0° and the crude PNA precipitated by addition of 2 vol of absolute ethanol previously cooled to 0°. Purified PNA was then obtained via its Ba salt by the method of MAGASANIK[4]. It was then hydrolysed with N HCl in a sealed glass tube and the products of the reaction (adenine, guanine, cytidylic acid and uridylic acid) separated on paper using the *t*-butanol/HCl/H_2O solvent of SMITH and MARKHAM[5]. The purine spots were eluted with 0.1 N HCl and the amount present determined spectrophotometrically; the eluates were then assayed for ^{14}C with a conventional end-window counter.

Results.

Ratio of guanine to adenine in R. rubrum PNA. Ten determinations on four different samples of purified *R. rubrum* PNA gave a mean value of 1.23 (range 1.15–1.34) for the molar ratio of guanine to adenine. This should be compared with values for the RNA of other bacteria, for example, 1.54, 1.47, and 1.14 for *S. marcescens*, *Mycobact. phlei* and *Escherichia coli*, respectively (ELSON and CHARGAFF[6]).

Incorporation of 8-^{14}C-adenine and 8-^{14}C-guanine into R. rubrum PNA. Representative results for 8-^{14}C-adenine and 8-^{14}C-guanine incorporation into *R. rubrum* PNA are given in the Table. From these results it is clear that *R. rubrum* falls into class b) discussed above, because it incorporates adenine into both PNA-purines, and guanine almost exclusively into PNA-guanine. This would suggest that if the generally accepted pathway for purine interconversions in micro-organisms (GOODWIN[2]) is also applicable to *R. rubrum*, then the enzyme carrying out the reductive deamination of guanylic acid (GMP to inosinic acid (IMP) is absent.

There is a very active adenine deaminase present in *R. rubrum* which, on a millimolar scale, converts adenine quantitatively into hypoxanthine which is itself not utilized by the organism (GOODWIN and PASSORN[7]). This

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accounts for the very low incorporation of 8-^{14}C-adenine (about 1%) into PNA compared with the incorporation of 8-^{14}C-guanine (about 50%) under the same conditions. Obviously with micromolar quantities, as used in these isotope experiments, small amounts of adenine, or hypoxanthine, do become incorporated; these would be undetectable with the concentrations used in the enzyme experiments.

Incorporation of 8-^{14}C-adenine and 8-^{14}C-guanine into *Rhodospirillum* PNA. (8-^{14}C-adenine added as adenine sulphate hemihydrate (S.A. 0.025 mC/mg) and 8-^{14}C-guanine added as the free base (S.A. 0.016 mC/mg). Each result is the mean of 4 determinations).

Counts added (cpm)	Counts in PNA (cpm)	Specific Activity (cpm/μM)		Ratio A/G
		Adenine	Guanine	
1. 195×10^3 (8-^{14}C-adenine)	2.09×10^3	4720	2440	1.94:1
2. 521×10^3 (8-C^{14}-guanine)	256×10^3	720	10400	0.07:1

Zusammenfassung. Das molare Verhältnis von Guanin zu Adenin in PNA von *R. rubrum* ist 1,23. Ganze Zellen von *R. rubrum* verbinden sich mit ^{14}C-Adenin in gleichem Mengenverhältnis zu PNA-Adenin und -Guanin, mit ^{14}C-Guanin aber fast ausschliesslich zu PNA-Guanin.

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Effect of some Hyper- and Hypo-Cholesteremic Drugs on the Cholesterol Biosynthesis by Liver Homogenates

There are some compounds that are capable of affecting the biosynthesis of cholesterol both *in vivo* and in liver slices, but whose mechanism of action has still not been identified.

Intravenous injection of Triton WR 1339 (arylalkylpolyether of phenol, Rohm & Haas, Inc., Philadelphia) has been shown to produce a rise in serum cholesterol[1,2] and FRANTZ and HINKELMAN[3] and BUCHER[4] found that hepatic cholesterol synthesis is accelerated 24 h after intravenous administration of Triton in rat.

Phenylethylacetic acid and its derivatives are known to produce a reduction in serum cholesterol of the hypercholesteremic animals and in the cholesterol biosynthesis of liver slices from acetate[5].

In order to ascertain whether any of these drugs may directly affect the metabolic pathways of cholesterol *in vitro* or whether they need the presence of the living cell, their effect on liver homogenates has been tested.

Materials and methods. A 30% rat liver homogenate containing the following in a volume of 2.5 ml, according to FRANTZ and BUCHER[6], is used: 0.08 *M* potassium phosphate buffer, pH 7.4, 0.03 *M* nicotinamide, 0.0048 *M* $MgCl_2$, 0.0008 *M* DPN, and 0.016 *M* sodium acetate. Three ml of the homogenate are distributed in two sets of 6 glass-stoppered 25 ml tubes. The first set (A) is added with 1 ml assay solution, the latter (B) with 1 ml water. All the tubes are sealed with 100% O_2 and maintained at 37°C for 2 h with shaking.

3 ml of the same homogenate are mixed in a third set of 6 tubes as control (C) with 1 ml water and 1 ml of assay solution.

Total cholesterol prior to incubation is determined according to Bloor.

At the end of incubation 1 ml of water to A and 1 ml of assay solution to B are added and immediately total cholesterol is determined from both the sets of tubes. pH values of all the tubes with and without assay solution at the end of experiment are controlled to be constant within $\pm$ 0.04.

Results. The mean cholesterol contents of liver homogenates in a typical experiment with Triton as assay solution are:

A) After incubation with Triton (final concentration in each tube 50 mg/ml): 249 mg%.

B) After incubation without Triton: 251 mg%.

C) Prior to incubation (control): 227 mg%.

Statistical analysis of the difference between the means B and C shows that incubation at 37°C stimulates a significant cholesterol biosynthesis from acetate in the liver homogenates at the rate of 10.6% (Student's t for 10 degrees of freedom = 3.69; $P < 0.01$). Triton is not capable to produce a rise in the rate of cholesterol biosynthesis at concentrations in the medium between 1 mg and 50 mg/ml.

Of the hypocholesteremic drugs phenylethylacetic acid (PEA), diphenylylethylacetic acid, 3-methyl-4-phenyl-3-butenoic acid (methylphenylvinylacetic acid[7]) and 2-methyl-4-phenylbutanoic acid (methylphenylbutyric acid[7]) have been tested.

The mean results of a typical experiment with sodium PEA as assay solution are: A) After incubation at 37°C with sodium PEA (final concentration in each tube 6 mg/ml): 237 mg%. B) After incubation at 37°C without sodium PEA: 240 mg%. C) Prior to incubation (control): 212 mg%.

Statistical analysis of the difference between the means B and C shows a significant Student's t (t for 10 degrees of freedom = 3.18; $P < 0.01$). In these experiments no inhibitory effect of PEA on the cholesterol biosynthesis by liver homogenates was evident at concentrations between 1 and 100 μM. Similar results were obtained in testing the other hypocholesteremic drugs mentioned.

Riassunto. Nè il Triton W R 1339 nè l'acido feniletilacetico sono capaci di modificare significativamente la biosintesi di colesterolo da parte di omogenati di fegato di ratto incubati a 37°C.

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