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Combination of Certain Dyes with Deoxycholic Acid.

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The present experiments were undertaken in the attempt to throw some light on the mechanism whereby certain water soluble dyes such as rose bengal, when injected intravenously into animals, pass through the liver and are excreted in the bile.[1] Rose bengal in particular, on account of this property, has come into use as a test for liver function.[2] The experiments of Wieland and Sorge[3] and others[4] showing that deoxycholic acid combines with fatty acids, xylol, phenol, camphor, naphthaline, cholesterol and related substances to form the corresponding choleic acid and that excretion of the conjugated compound via the bile constitutes a method whereby the detoxicated product is eliminated, suggested that rose bengal might also combine with deoxycholic acid. The preparation of the rose bengal-deoxycholic acid complex was therefore undertaken.

Varying quantities of rose bengal were heated for several hours under a reflux condenser with known amounts of an alcoholic solution of deoxycholic acid (250 mg. of deoxycholic acid dissolved in 5-10 cc. of alcohol), an excess of dye always being present. The mixture was then concentrated *in vacuo* until a semi-viscous fluid was obtained. The presence of deoxycholic acid increases the solubility of the dye in alcohol. On addition of water the deoxycholic acid-dye compound was precipitated and the excess of dye was removed by repeatedly washing with water. The compound was recrystalized several times from alcohol containing a little rose bengal by addition of water. The excess of dye was removed by washing with water. The amount of dye contained in the complex was

[1] Brakefield, J. L., and Schmidt, C. L. A., PROC. SOC. EXP. BIOL. AND MED., 1926, **23**, 583.

[2] Delprat, G. D., *Archiv. Int. Med.*, 1923, **32**, 401. Delprat, G. D., Epstein, N. N., and Kerr, W. J., *Arch. Int. Med.*, 1924, **34**, 533. Kerr, W. J., Delprat, G. D., Epstein, N. N., and Dunievitz, M., *J. Am. Med. Assn.*, 1925, **85**, 942.

[3] Wieland, H., and Sorge, H., *Z. physiol. Chem.*, 1916, **97**, 1.

[4] Rheinboldt, H., *Ann. d. Chem.*, 1926, **451**, 256. Rheinboldt, H., König, D., and Flume, E., *Z. physiol. Chem.*, 1929, **184**, 219. Osten, F. W. R., and Grube, E., British Patent 287965, Dec. 30, 1926, quoted after *Chem. Abs.*, 1929, **23**, 484. Schönheimer, R., *Biochem. Z.*, 1924, **147**, 258. Schmidt, W., and Schmidt, C. L. A., *Univ. Calif. Pub. Physiol.*, 1930, **7**, 211.

estimated colorimetrically by comparison with a standard solution of rose bengal containing 1 mg. in 10 cc. of alcohol. The dye could be dissociated from the compound by boiling with xylol.

TABLE I.

Amount of Dye Added		Dye found	Calculated	Proportion of dye to deoxycholic acid
mg.		%	%	
100	Tetrachlortetraiodo-fluorescein	20	19.9	1:10
75	" "	19.8		
60	" "	20.5		
50	" "	20.1		
75	Trichlortetraiodo-fluorescein	13.6	13.8	1:15
50	" "	14.7		
50	Tetrachlor-fluorescein	7.3	7.4	1:15

Table I gives the results obtained. The data indicate that the various rose bengal dyes form definite compounds with deoxycholic acid. Contrary to expectations no compound was formed when tetrachlorphthalein was similarly treated with deoxycholic acid.

Further evidence of compound formation between deoxycholic acid and rose bengal was obtained from conductivity estimations, carried out on solutions containing either 400 mg. of the complex or equivalents of deoxycholic acid, dye or mixtures of the latter. The specific conductivity data are: deoxycholic acid-rose bengal complex (400 mg. per 50 cc. of 95% alcohol), 7.75×10^{-6}; rose bengal (80 mg. per 50 cc. alcohol), 8.68×10^{-5}; deoxycholic acid (320 mg. per 50 cc. alcohol), 6.64×10^{-7}; mixture (80 mg. rose bengal + 320 mg. deoxycholic acid per 50 cc. alcohol), 8.47×10^{-5}.

Attempts to form similar compounds of deoxycholic acid with the fat soluble dyes, sudan III and scharlach R, which are eliminated in both bile and urine,[5, 6] gave negative results. These dyes could be almost completely removed by washing with ether subsequent to treatment with deoxycholic acid. The observations of Salant and Bengis[5] and of Joel and Schönheimer[6] point to the probability that the portion of these dyes which appears in the urine after administration is conjugated with glycuronic acid. The solubility of sudan III, as Joel and Schönheimer have pointed out, is markedly increased by the presence of sodium deoxycholate, a factor which may be concerned in the elimination of some of the dye in the bile.

We have observed when stearin choleic acid was treated with either sudan III or scharlach R in a manner similar to that used for the synthesis of the rose bengal-deoxycholic acid compound that it

[5] Salant, W., and Bengis, R., *J. Biol. Chem.*, 1916, **27**, 403.

[6] Joel, E., and Schönheimer, R., *Centralbl. f. allgemein. Path.*, 1924, **34**, 625.

was not possible to wash the stearin choleic acid entirely free from dye by the use of ether. When the stearin choleic acid was decomposed by boiling with xylol, the dye was set free. The content of dye was about 1%. When stearin choleic acid was treated with rose bengal all of the dye could be removed by washing with water. The results suggest that possibly a compound was formed which contained both stearic acid and fat soluble dye.

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Localizations of Poliomyelitic Virus During Incubation Period after Intranasal Instillation in Monkeys.*

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During the interval, corresponding with the incubation period in man, between intranasal inoculation of poliomyelitic virus in the monkey and the onset of spinal symptoms, the localizations of the virus are, with one exception, unknown. The exception is the olfactory bulb, where Flexner and Clark[1] found virus 48 hours after application to the nasal mucosa. At that time none was demonstrable in the medulla or spinal cord. In the present study, *Macacus rhesus* monkeys were given 3 intranasal instillations, without trauma, of active MV virus at intervals of 3 hours, or within a total period of about 6 hours. Monkeys so treated were sacrificed on the third, fourth, fifth, sixth, and seventh days, respectively. Brain and spinal cord were removed under aseptic precautions. Specimens were removed from various parts of the central nervous system, ground and suspended in physiological salt solution to approximate 10% concentration and injected intracerebrally in monkeys in 1.5-2.0 cc. amounts. These animals were observed for the appearance of the usual signs of poliomyelitis and, if positive, were examined postmortem for gross and histological lesions.

The efficacy of the intranasal method of inoculation employed was shown by a series of 26 monkeys used in a separate study (unpublished) by Schultz and Gebhardt, in which 23, or 88%, devel-

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[1] Flexner, S., and Clark, P. F., PROC. SOC. EXP. BIOL. AND MED., 1912, **10**, 1.