S colonies from an initial R form, and R forms from an initial S colony again, developed after a subsequent reculturing of the second dissociants. Obviously, either the homologous colony was not homogeneous for type or spontaneous dissociation was taking place.

To answer this question several single bacteria were isolated from both the R and S types of colonies. The single bacterial cells were allowed to form micro-colonies in the micro-culture after which the resultant growth was drawn into a micro-pipette and expelled upon a slant of Petroff's egg medium. By allowing a micro-colony to form in the microscopic drop containing a single bacterial cell before any attempt is made at transplantation, a much higher percentage of successful cultures is obtained from single cells than if the freshly isolated single organism is planted directly. The details of this technic will be published later.

The cultures obtained from single organisms of both the R and S types of colonies bred true for 3 or more generations, giving rise only to their homologous colony forms. Sooner or later, however, in both instances dissociation unquestionably occurred. Typical R colonies grew on plates receiving inoculation with an actual S colony from a single cell S strain and S colonies developed on plates which were seeded with an actual R colony from an R single cell strain. The mutation is somewhat more prolific from S to R, but the R to S change may not be termed at all infrequent, as is the case among many other species of bacteria. The dissociation of these single cell strains took place spontaneously, no special effort being made to induce it. This confirms observations on single cell cultures of avian *Mycobacterium tuberculosis*.[7]

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Relative Immutability of Hydrogen Ion Concentration of the Bile. Its Buffering Effect in Bactericidal Experiments.

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In 1926 we noticed that solutions of phenol-tetra-chlor-phthalein and phenol-tetra-iodo-phthalein are decidedly bactericidal for typhoid bacilli. These dyes, however, when concentrated in the gall-bladder of animals after intravenous injection (Abel and Rown-

[7] Kahn, Proc. 33rd Ann. Meeting Soc. Am. Bact., 1931, 10.

tree[1]) show no bactericidal effect whatever. In simple experiments we found that the bactericidal effect of aqueous solutions of these dyes was due entirely to their alkalinity. As excreted, however, they seem to be in a colloidal state and have no effect on the reaction of the bile.

We wished, therefore, to find out whether it was actually possible to make the bile more alkaline. A Rous fistula[2] was established in a dog, the bile collected and examined daily by potentiometer. It was impossible to produce an appreciable change in the reaction of the 24 hour bile by administration of large doses of sodium bicarbonate by mouth or intravenously, or by intravenous administration of the very alkaline solution of phenol-tetra-chlor-phthalein (pH 9.52). (Protocol I.) There was not even a transient increase in alkalinity of bile after the injections; a specimen collected for 6 hours after an intravenous injection of bicarbonate of soda showed a change toward the alkaline side too small to be of significance.

PROTOCOL I.

Dog 1 Male, 10 kilo wt. Procedure	pH of 24 hour bile	Day after operation	pH of 24 hour bile	Dog 2 Female, 9 kilo wt. Amt. of bile in 24 hrs.	Procedure
	8.65	2	8.30	60 cc.	
	8.90	3	8.60	55	
After 10 gm. $NaHCO_3$ by stomach tube	8.92	4	8.55	70	
	8.69	5	8.45	70	After 20 gm. $NaHCO_3$ by stomach tube
		6		(2 days)	
		7	8.35	210	
After 12 gm. $NaHCO_3$ by stomach tube	8.66	8	8.36	110	
		9	8.40	90	
1.5 gm. $NaHCO_3$ (25 cc. of 5% solution	8.75 (6 hr. spec.)	11	8.40 (4 hr. spec.)		30 gm. $NaHCO_3$ by stomach tube*. Spec. examined, 10 cc. collected 4 hr. after bicarbonate administration
Intravenous injection of 5 cc. of 5% phenol-tetra-chlor-phthalein	8.45	15			
	8.60	16			
	8.60	22			
	8.60	23			

* Vomited a small amount after 1½ hours.

[1] Abel and Rowntree, *J. Pharm. and Exp. Therap.*, 1909, **1**, 231.

[2] Rous and McMaster, *J. Exp. Med.*, 1923, **37**, 11.

Recently we became interested in attempts to cure typhoid carriers. Nichols,[3] hoping to cure typhoid carriers, attempted to make the patient's bile more alkaline by administration of large doses of sodium bicarbonate by mouth. (Typhoid bacilli are easily killed by alkaline solutions.) Some favorable results were reported. Krause[4] states that "change of reaction by administration of acids or alkalies has so far given no results".

We therefore repeated our earlier animal experiment to make the bile more alkaline. We found it was impossible to make the bile more alkaline. (Protocol I, Dog 2.)

Duttmann[5] gave large doses of alkalis or acids by mouth to patients with temporary biliary fistulas. He found only negligible changes of hydrogen ion concentration of the bile. We have confirmed Duttmann's results in experiments on 3 patients convalescent after the operation of cholecystectomy with drainage of the common bile duct. The daily drainage of bile was collected with sterile precautions and its hydrogen ion concentration determined by potentiometer. Oral administration of as much as 20 gm. of sodium citrate or 24 gm. of sodium bicarbonate or of 6 cc. of dilute hydrochloric acid failed to cause any significant change in the pH of the bile (Protocol II).

PROTOCOL II.

Day after operation	Patient	Administered to patient *(per os)*	pH of Bile Pat. A	Pat. B	Pat. C
2			8.11	8.61	8.30
3			8.07	8.53	8.20
4	A	Sodium citrate 4 gm. every 4 hr. 5 doses. 24 hr. bile	**8.11**	8.30	7.84
5				8.20	
6	B	$NaHCO_3$ 12 gm. in 1 hr. (divided doses). Bile in 3 hr. after bicarbonate	8.31	**8.62**	8.04
7	A	$NaHCO_3$ 4 gm. every 4 hr. for 6 doses. 24 hr. bile	**8.20**	8.61	
	C	Sodium citrate 12 gm. within 1 hr. 3 hour bile collection			**8.14**
8				8.62	7.80
9	B, C	Dilute HCl 6 cc., divided doses, 1 hr. 3 hour bile collection		**8.56**	**7.87**
			Bile very dark	Bile throughout very dark viscid	Bile throughout rather pale and fluid

[3] Nichols, *J. Am. Med. Assn.*, 1917, **68**, 958.

[4] Krause, *Monatschr. f. Kinderheilkunde*, 1929, **44**, 358.

[5] Duttmann, Bruns, *Beitr. z. klin. Chir.*, 1927, **139**, 720.

It is interesting to note that the bile of each dog and of each patient tends to range about a somewhat characteristic average pH. The bile of the dogs was sterile or nearly so. That of the patients was undoubtedly infected to some degree. This may perhaps explain the greater variability of the patient's pH.

The bactericidal effect of alkali added directly to bile is nullified by the buffering effect of bile. This was shown by the following experiment. In 2 rows of tubes graded quantities of N 1/10 sodium carbonate (0.1, 0.2, 0.3, 0.4, 0.5 cc.) were diluted to equal volume (1.5 cc.) using as diluent in the first row 1 cc. of sterile (boiled) human fistula bile and a small amount of water, in the second row, water only. Then 0.1 cc. of diluted typhoid culture in bouillon was added to all the tubes. After one hour incubation at 37°C. the contents of all were plated in agar. Even the largest amounts of sodium carbonate, when mixed with bile, failed to show any bactericidal effect, while even the smallest amount showed some bactericidal effect in water.

This effect is not caused by any protective effect of bile for the bacteria. We have tested bile with a number of antiseptics, $HgCl_2$, mercurochrome, salyrgan, and others, and found it only to interfere slightly with their bactericidal power for typhoid bacilli. We have commenced work on the titrimetric buffer effect of bile.

Summary. It thus appears that (1) alkalis given by mouth or vein and hydrochloric acid by mouth produce hardly any change in the hydrogen ion concentration of secreted bile and (2) the bactericidal effect of alkaline solutions is destroyed by addition of bile. (3) Increased alkalinization of bile (at least by the methods used) does not occur. The great stability of the hydrogen ion concentration of bile is a fact of considerable interest.

This observation, together with the well recognized fact that increased fluid intake does not increase the volume of bile secreted[6,7,8] suggests that bile secretion is relatively independent of those conditions of water and electrolytes in the blood which so greatly influence the secretion of urine.

[6] Westphalen, 1873.

[7] Gundermann, *Beitr. z. klin. Chir.*, 1923, **128**, 1.

[8] Snell and Rowntree, *Am. J. Physiol.*, 1928, **85**, 577.