Brit. J. Pharmacol. (1950), **5**, 1.

23148

A STUDY OF THE EFFECTS AND MECHANISM OF ACTION OF SODIUM DODECYL SULPHATE ON GASTRIC SECRETION IN RATS

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(Received October 17, 1949)

In 1942 sodium lauryl sulphate, a synthetic detergent of the anionic series, was reported by Shoch and Fogelson (1942a) to inactivate pepsin *in vitro* without altering the hydrogen ion concentration of the solution. They had previously found (1942b) a greatly prolonged survival time in dogs treated with this detergent in whom ulcers had been experimentally induced by histamine (Varco, Code, Walpole, and Wangensteen, 1941). In 1944 Fogelson and Shoch described good clinical results after the administration of sodium lauryl sulphate to patients whose ulcers appeared to be resistant to other forms of therapy.

The *in vitro* results reported by Shoch and Fogelson have been confirmed. Kirsner and Wolff (1944) studied a number of anionic detergents and found the alkyl sulphates most effective in inhibiting the peptic activity of human gastric contents; and of these decyl (C_{10}) and dodecyl (C_{12}) derivatives were the most active. Kirsner and Spitzer (1944) demonstrated that the inactivation of pepsin by sodium alkyl sulphate is an irreversible process.

In vivo, however, the findings reported by Shoch and Fogelson were not corroborated by others. Kirsner and Wolff (1943) observed that inhibition of pepsin by sodium alkyl sulphate was impeded *in vitro* by cream, butter lecithin, glycerin, and esters of fatty acids. Even with large doses of sodium alkyl sulphate they encountered difficulty in lowering peptic activity in patients fed low-fat diets. What inhibition of peptic activity they did observe was usually temporary and without beneficial effect. Similarly discouraging clinical results with sodium lauryl sulphate were reported by Steigmann and Marks (1943). It is apparent from the literature that further basic studies are necessary before these agents can acquire a place in the therapy of gastric and duodenal ulcer.

We have experimented with a number of alkyl sulphates with an even number of carbon atoms from C_8 to C_{18}. We selected sodium dodecyl sulphate, hereafter referred to as SDS, for detailed study because this member of the group was used largely in previous investigations and especially because *in vitro* it inhibited peptic activity to a degree equalled only by the decyl (C_{10}) compound.

SDS introduced into the stomach had been found capable of marked and manifold effects upon gastric secretion. In the dog a 2 per cent (w/v) solution exerted a powerful mucigogue action and inhibited parietal cell secretion, even after massive doses of histamine (Shay, Komarov, Siplet, and Fels, 1946). In the rat (Shay, Komarov, Siplet, and Gruenstein, 1947) a similar mucigogue effect was obtained with a wide range of concentrations of SDS, but the nature of its action on the parietal cell—inhibition or stimulation—appeared to be dependent on the concentration of the drug.

In addition to studying the physiological mechanism involved in the action of SDS, we have been able to relate the type of gastric secretory response in the rat to the concentration of SDS employed.

METHODS

White rats of the Wistar strain weighing 150 to 180 grammes, grown in our own colony, were used for the study. Light urethane narcosis (0.75 c.c./100 g. of a 5 per cent (w/v) solution intracaecally) supported by ether was maintained only during the operation. The animals recovered rapidly from the anaesthetic and therefore were under narcosis only during a small part of the time of actual experiment. In animals fasted for 48 hours a mid-line abdominal incision was made. A small portion of the caecal wall was exteriorized and the pylorus ligated. The oesophagus was exposed in the neck and lifted on a loose ligature without damage to blood vessels or adjacent nerves. The stomach was washed out once with 4 c.c. of warm normal saline through a catheter inserted through the mouth and withdrawn immediately. In many instances the full 4 c.c. were removed, but the catheter was nevertheless reintroduced to empty the stomach just before the introduction of the test solution. Removal and reintroduction of the catheter for each operation was found necessary. Under anaesthesia the rat tolerated repetition of gastric intubation but rapidly became cyanotic if the catheter remained in place for more than 30 seconds. If the lavage fluid was more than slightly cloudy on recovery, the animal was discarded. Following this procedure groups of animals were treated in various ways:

GROUP B:—basic group—oesophagotomy only. After 2 c.c. of the test solution had been introduced into the stomach, the oesophagus was ligated in the neck as the catheter was withdrawn. The oesophagus was sectioned just cephalad to the ligature, and the cut end of the upper segment was brought to the surface and fixed by sutures without encroaching upon the lumen. The abdominal wound was closed.

GROUP BA:—The animals were operated upon as in Group B. Twenty-five mg. of atropine sulphate per 100 g. body weight were injected subcutaneously at the beginning of the operation.

GROUP BV:—In this group, in addition to the procedure followed in Group B, a loose ligature was placed around the lower end of the oesophagus just above the cardia. Care was exercised to include both vagi in the ligature and to avoid any injury to blood vessels. After 2 c.c. of the test solution were instilled into the stomach through the catheter, the ligature was tied firmly as the catheter was withdrawn. The abdominal wound was then closed.

GROUP BVA:—Procedure of Group BV was used and atropine was injected as in Group BA.

Each experiment was terminated six hours after the gastric instillation of the test substance. The gastric contents of every animal were collected and measured

individually in graduated centrifuge tubes and centrifuged for 10 minutes at 3,000 revolutions. The supernatant and sediment were separated. The volume and gross description of each fraction were recorded. The volume of gastric juice per hour per 100 g. of rat was calculated after 2 c.c. (volume instilled) were deducted from the recovered volume. When the volume of supernatant was adequate, the following determinations were made by methods routine in our laboratory (Shay *et al.*, 1947): free and total acidity, *p*H, total chloride, pepsin, and mucin.

SDS was used in this study in solutions of 0.1, 0.5, 1, and 2 per cent (w/v) concentration prepared in distilled water. Two control groups were used—one with distilled water alone, the other with a sodium sulphate solution. The latter group was necessary because in acid media the alkyl sulphates are readily hydrolysed with the liberation of inorganic sulphates. The concentration of sodium sulphate solution used was 0.066 per cent (w/v) which was calculated to be approximately isosmotic with concentrations of SDS of 0.2 per cent (w/v) and greater. Hess and Suranyi (1939) have shown that SDS exists in true solution only in concentrations up to 0.2 per cent (w/v) (6.95 mM/l.). Higher concentrations result in the formation of micelles which do not contribute materially to the osmotic pressure.

RESULTS

Results obtained for each animal are charted in the distribution graph (Fig. 1), while mean values for the respective groups are presented in the Table.

TABLE

THE MEAN VALUES FOR THE RATE AND COMPOSITION OF GASTRIC SECRETION IN RESPONSE TO THE INSTILLATION OF DISTILLED WATER, SODIUM SULPHATE, AND SODIUM DODECYL SULPHATE

	Description		No. of animals	Rate c.c./hr./100 g.	*p*H	Acid (mEq./litre) Total	Acid (mEq./litre) Free	Cl⁻ mEq/l.	Pepsin Mett units	Mucin mg./100 ml.	Mucin mg./hr./100 g.	Gross appearance of gastric specimens
Distilled Water		B	10	−0.07	2.06	25	13	71	36	283	0.5	All clear
		BVA	11	−0.13	2.82	17	2	49	10	367	0.5	,,
Sodium Sulphate	0.066%	B	8	−0.06	1.92	32	19	79	31	243	0.5	,,
		BV	13	−0.06	2.35	17	7	61	22	172	0.4	,,
		BA	11	−0.09	2.88	10	2	33	1	324	0.5	,,
		BVA	11	−0.07	2.95	11	1	36	3	256	0.5	,,
Sodium Dodecyl Sulphate	0.1%	B	9	+0.30	1.53	47	31	138	105	474	2.2	All turbid
		BVA	10	−0.09	2.74	27	5	122	2	783	1.8	1 clear (*p*H 6.36), 9 turbid (*p*H <4.2)
	0.5%	B	9	+0.53	1.57	42	31	143	38	436	3.5	All turbid
		BV	13	+0.06	6.76	6	0	120	0	432	1.8	All clear
		BA	11	+0.02	7.08	4	0	103	0	439	1.2	,,
		BVA	10	+0.04	5.45	7	0	120	0	758	2.6	,,
	2.0%	B	8	+0.44	6.41	6	0	110	0	832	5 7	,,
		BV	13	+0.24	7.09	5	0	105	0	651	3.4	,,
		BA	12	+0.09	7.23	5	0	103	0	680	2.3	,,
		BVA	10	+0.17	7.24	3	0	106	0	750	3.3	,,

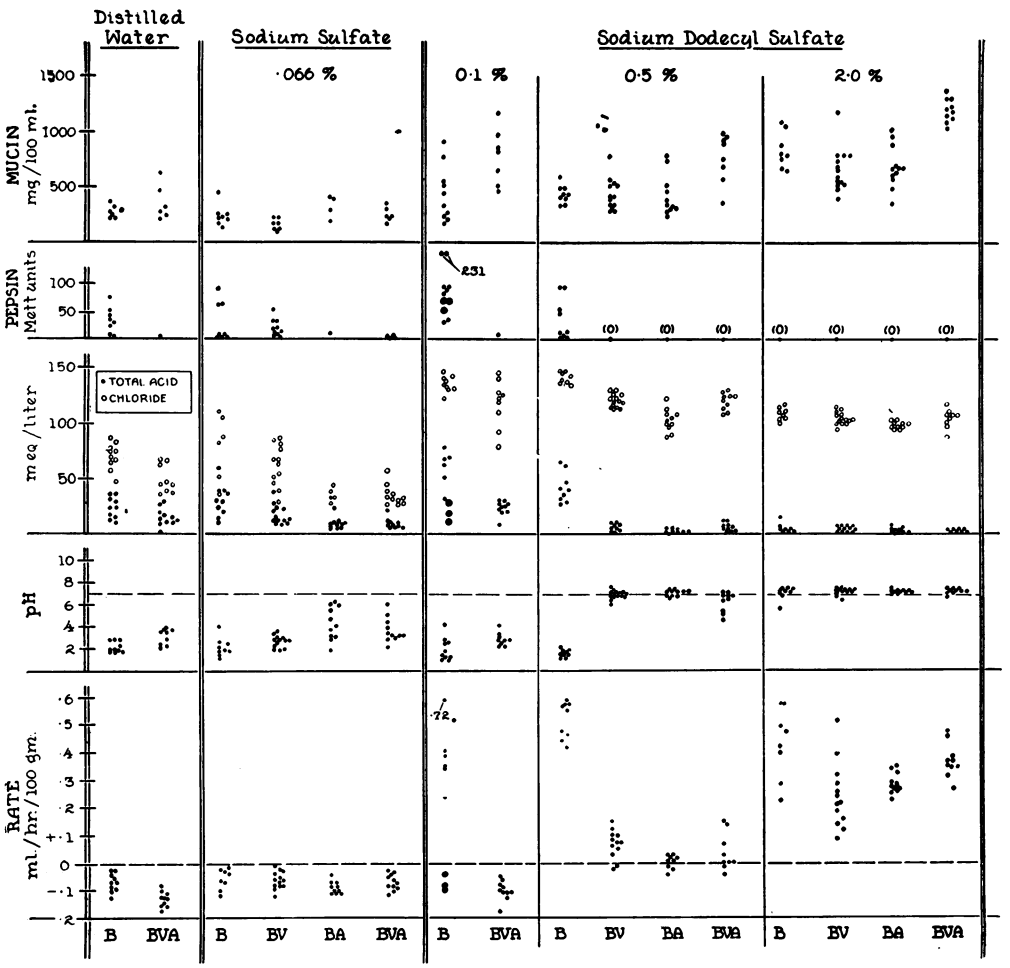

FIG. 1.—Distribution chart of the effect of intragastric instillations of SDS solutions in comparison with that of distilled water and Na_2SO_4 solutions. Groups B, BV, BA, and BVA as described fully in *Methods*. B (basic group), oesophagotomy plus pyloric ligation. BV, same as B plus vagotomy. BA, same as B plus atropine sulphate. BVA, same as B plus vagotomy plus atropine sulphate.

Effect of sodium dodecyl sulphate solutions.—SDS always produced a marked effect on gastric secretion, the nature and magnitude of which depended upon the concentration of the solution. In the lower concentrations, from 0.1 to 1 per cent, SDS stimulated secretion of all the basic constituents of the gastric juice, acid, pepsin, and mucin, whereas in concentrations of 2 per cent and higher it stimulated secretion of mucus alone. These two types of response were reflected in the gross appearance and physical properties of the gastric contents recovered. When all glandular elements were stimulated, the gastric contents were turbid and contained varying amounts of greyish-white precipitate mixed with clumps of

viscous mucoid material. The fluid remained highly opalescent, even after prolonged centrifugation. The turbidity and the appearance of the precipitate were due to the interaction (in acid medium) of SDS and pepsin. The *p*H of these samples ranged from 1.2 and 4.3. When mucus alone was secreted, the contents were clear and exceedingly viscous. These observations are in accord with the results obtained by Putnam and Neurath (1944); they found the inhibition of pepsin by SDS to be due to precipitation of the enzyme which is maximal at the isoelectric point for pepsin, *p*H 2.5. At a *p*H greater than 5.5, SDS fails to cause any precipitation.

The effects of SDS in the lower concentrations are illustrated in Fig. 1 and in the Table in Group B (0.1 and 0.5 per cent). The results with 1.0 per cent solutions, which are not recorded, were very similar to those obtained with 0.5 per cent solutions. The rate of secretion, acidity, and the concentration of pepsin and mucin are very much higher than in the control series with distilled water or sodium sulphate. Stimulation of the parietal cells is also reflected in a considerable increase in total chloride concentration and a lower *p*H. On the whole, the secretagogue effect of 0.5 per cent solution was considerably more pronounced than that of 0.1 per cent solution. Pepsin, however, showed a concentration and an output which were much lower with the 0.5 per cent than with the 0.1 per cent solution.

It is probable that the analytical data concerning pepsin do not truly reflect the degree of stimulation of the peptic cells. It is conceivable that stimulation of the peptic cells by 0.5 per cent SDS was actually greater than with 0.1 per cent, but the increased pepsin output could be masked by the greater inhibitory effect on the enzymatic activity of the 0.5 per cent solution of the detergent.

Solutions of SDS of 2 per cent or greater produced the clear viscous type of gastric secretion. Inspection of the analytical data obtained with the 2 per cent solution will reveal (1) that the rate of secretion was still very high but a little lower than with 0.5 per cent SDS; (2) that the *p*H of the contents in the majority of the animals was alkaline (Fig. 1); and (3) that there was no detectable peptic activity. On the other hand, the concentration of mucin was about twice as high as with the 0.1 or 0.5 per cent solutions of SDS. Other characteristics of the gastric contents—namely, the extremely high viscosity and the typically low total chloride values—suggest that the gastric secretion was pure mucus in all experiments except one in which the *p*H was in the acid range.

The absence of parietal cell secretion with the 2.0 per cent solution is due, we believe, not only to a failure of the SDS in this concentration to stimulate the parietal cells but actually to the inhibition of their activity. We have seen a striking and prolonged inhibition of the secretory response to massive doses of histamine in dogs after the gastric instillation for only 30 minutes of a 2.0 per cent SDS solution (Shay *et al.*, 1946). The effect upon the peptic cells of the 2.0 per cent solution cannot be as clearly defined at present. The assumption might be justified that secretion of pepsin, like that of acid, may be inhibited. Failure to detect peptic activity in the mucus samples obtained with the 2.0 per cent solution cannot be offered as evidence *per se*. In the majority of the specimens

in this group the high *p*H of the gastric contents alone could have destroyed the secreted pepsin, although this should not have occurred in two of the specimens which had *p*H values as low as 5.6. However, since any method for pepsin determination requires acidification of the specimen to an optimal *p*H, a precipitation and/or inhibition by the detergent could occur during the process of pepsin determination.

While the rates of secretion observed with 0.5 and 2.0 per cent SDS appear to be low (0.53 and 0.44 c.c./100 g.), they are actually very high for the rat. Compared on the basis of relative weights, such rates would represent in a 70 kilo man an hourly secretion of 370 and 310 c.c., respectively.

We have not determined the exact range of concentrations within which SDS would produce one or the other type of gastric secretory response. The response to the 0.1 per cent solution was not uniform (Fig. 1 and Table, Group B); three rats of the group of ten did not show any increase in gastric secretion over the control values. The response to the 0.5 per cent solution was uniform. The 2 per cent solution produced wide divergencies in the rates but not in the composition of mucus secretion in the different animals. It is also significant that the mucigogue effect produced in the rat by this concentration was similar to that previously reported in the dog (Shay *et al.*, 1946). Furthermore, a close similarity in the composition of the mucus of the two species was found.

Mechanism of secretagogue action of sodium dodecyl sulphate.—A comparison of results obtained with 0.5 per cent solutions of SDS in the non-atropinized (B) and atropinized (BA) animals (Fig. 2 and Table) shows that atropine completely abolishes the stimulating action of SDS on the parietal cells. Group BA exhibited a considerable diminution in the mucigogue effect. With the 2.0 per cent solution of SDS which stimulated only the mucous cells, atropine decreased the rate without altering the composition of the secretion. Ligation of the lower end of the oesophagus, including both vagus trunks (Group BV), largely nullified the effect of SDS on the parietal and peptic cells. Supplementary treatment with atropine (Group BVA) in such animals produced no further change in *p*H, acidity, chloride, or pepsin concentration, but lowered the rate of secretion; mucin concentration, however, was increased. From these results it is evident that the secretagogue action on parietal and peptic cells by SDS in 0.1 or 0.5 per cent solutions is entirely reflex in nature, the impulses being transmitted through the vagi. In the experiments with 0.5 and 2 per cent SDS, mucin output was only partially inhibited by atropine and by ligation of vagi. This indicates that the mucigogue action of SDS was only, in part, reflex by way of the vagus.

Although experiments with distilled water and sodium sulphate solution were performed primarily as controls, they revealed certain facts which are of considerable interest in themselves.

Effect of distilled water.—In this series (Fig. 2) less fluid was recovered from the stomach after six hours than was originally instilled, the decrease being due to absorption. Although some investigators still question any absorption by the stomach, there is considerable evidence for the ability of the stomach to absorb

hydrochloric acid (Ihre, 1939; Teorell, 1939). One of us (H.S.) has demonstrated the absorption of glucose under certain conditions by the stomach of man (Shay, Gershon-Cohen, Fels, and Munro, 1939) and of the dog (Morrison, Shay, Ravdin, and Cahoon, 1939). In our present experiments absorption by the stomach is especially well manifested in the animals which had received atropine (series BA and BVA). The rate of absorption in such animals (average 0.13 c.c./hour/100 g. body weight) appeared to be nearly twice that of animals which did not receive atropine (average 0.07 c.c./hour/100 g. body weight). This may represent a difference in the rate of concurrent gastric secretion rather than in gastric absorption in these two groups, since water produces a higher secretion in the non-atropinized than in the atropinized rat. This is shown by the difference in the rate and also by the lower *p*H and higher values for acid, chlorides, and pepsin in the gastric contents found in the non-atropinized series. In a study of large series of rats, to be reported elsewhere, we found that the dose of atropine used will usually inhibit the spontaneous gastric secretion during the six-hour experimental period.

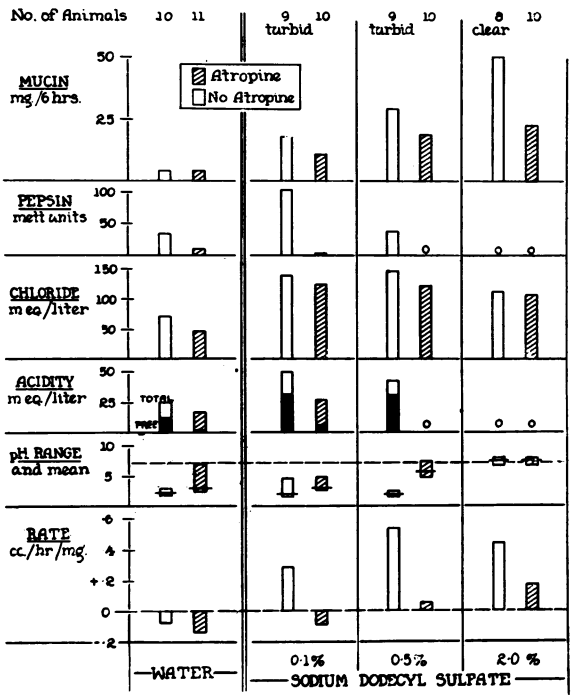

FIG. 2.—The effect of distilled water on gastric secretion in rats with oesophagotomy and pyloric ligation. All barographs represent the mean values from all experiments in respective groups. Free acidity, black.

Effect of sodium sulphate solution.—The sodium sulphate solution produced a gastric response which did not differ significantly in the rate of secretion or composition of the contents from that obtained with water alone.

COMMENT

From an analysis of our results it is apparent that under certain conditions SDS applied in sufficiently high concentrations in an empty, fasting stomach may produce a secretion of mucus while actually inhibiting parietal secretion and peptic activity. These two effects would be very desirable in the management of gastric and duodenal ulcer. However, when SDS is present in the stomach in dilute solution, certain undesirable effects may result from a stimulation of secretion of acid and pepsin. This was demonstrated in a previous paper (Shay

et al., 1947) showing the influence of SDS upon the experimental production of ulcer in the rumen of rats. The divergence of results obtained by various investigators in the treatment of ulcer patients with SDS may be due to the lack of control over the final concentration of the agent in the stomach.

SUMMARY

When sodium dodecyl sulphate is introduced into the isolated stomach of a fasting rat, the response of the gastric secretory mechanism is related to the concentration of the agent. With 0.1 and 0.5 per cent (w/v) solutions all the secretory elements—the parietal, peptic, and mucous secreting cells—are strongly stimulated. The 2.0 per cent (w/v) solution exerts a selective effect, activating only the mucus secreting cells. The response of the parietal and peptic cells to stimulation by weak solutions of sodium dodecyl sulphate is entirely a reflex effect through the vagi. However, stimulation of the mucus secreting cells is due only in part to such a reflex effect.

We are indebted to E. I. du Pont de Nemours and Company, Wilmington, Delaware, for generous supplies of sodium dodecyl sulphate.

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