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THE EFFECTS OF RESTRAINT ON UPTAKE OF RADIOACTIVE SULFATE  
IN THE SALIVARY AND GASTRIC SECRETIONS OF RATS WITH PYLORIC LIGATION

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EFFECTS OF RESTRAINT ON THE INCORPORATION OF RADIOACTIVE SULFATE  
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J. A. Chayvialle, R. Lambert and D. Ruet

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Restraint provokes in the rat a diminution of sulfated mucous substances present in the glandular portion of the gastric wall. A relationship between this variation and ulcerogenesis has been suggested [1,2]. Radioactive sulfate is incorporated specifically in the form of ester-sulfates in these mucous substances. The reduction of this incorporation in the restrained animal can be due either to a decrease of the synthesis of the ester-sulfates in glandular mucosa, or to an increase in their excretion into the gastric juice. Simultaneous study of the radioactive sulfate in the wall and in the contents of the stomach should make it possible to decide between these two hypotheses. It is necessary, however, not to exclude contamination of the gastric juice by saliva. This work is dedicated to the study of the effects of restraint on the amount of non-dialysable radioactive sulfate in the gastric wall, in the gastric juice and in saliva. Various procedures have been employed in order to collect these two secretions separately.

Method. 102 Wistar rats (86 males, 180-200gm, and 16 females, 200-250 gm) received at the beginning of the experiment 100  $\mu$ C of sulfur 35 by the i.p. route (sodium sulfate, C.E.A.). They were divided into three groups according to the surgical protocol which always included pyloric ligation by the Shay technique [3]: Group I: gastric secretion was collected in 60 rats, either after isolated /115 pyloric ligation (group 1.a: 29 animals), or after pyloric and eso-

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phageal ligation (group I.b: 31 animals), thus eliminating contamination from saliva. The contents of the stomach and the gastric glandular wall were analyzed simultaneously. Group II: in 16 rats a ligature was placed the length of the border between the rumen and the glandular stomach. The salivary secretion thus was collected in a pure state in the rumen. Group III: in 26 rats, the saliva was collected directly by means of a catheter maintained in the abdominal esophagus by an esophageal ligature and connected to an intraperitoneal small balloon. In each group, the animals were strictly paired according to weight and sex: a control in the cage, the other rat restrained in a metallic tube [4].

The rats were sacrificed with ether at the 6th hour. The stomach or the rumen were opened and washed with 20 ml of 0.3M sodium chloride (pH = 1.5). The gastric glandular wall was separated from the rumen and ground up. About 1/10 of the tissue was sampled for determination of relation of the dry weight to the fresh weight (weighed before and after dessication at 100°C for 24 hours). The remainder of the glandular wall was placed in 20 ml 0.3M sodium chloride (pH = 1.5) and subjected to the same analysis time as the secretions: proteolysis by pepsin (Worthington twice-crystallized pepsin, 20 mg per sample, 24 hours at 37°C), dialysis in running water for 48 hours after addition of 2 ml 5% sodium sulfate for comparison (laboratory temperature). The radioactivity was determined at the end of the dialysis by a liquid scintillation counter (Packard Tricarb 3375). After correction for quenching and loss, the results were expressed in dpm. The standard deviation of the average was calculated for each value and indicated in the tables. Non-dialysable radioactive sulfate after peptic proteolysis is considered the index of incorporation of the isotope in the sulfated glycoproteins and polysaccharides. The different series were compared by the Student t test, the threshold of probability being fixed at 5%.

Results. - 1. When the gastric contents are collected after isolated pyloric ligation (group I.a.), the restraint provokes a significant decrease ( $p < 0.05$ ) of volume and of the quantity of

radioactive sulfate in the gastric contents (18.4% of the value for the controls). Incorporation of the isotope in the wall decreases but the change is not significant (total amount: 82.5% of the control value).

2. When an esophageal ligation was performed in the rats with pyloric ligation (group I.b), the quantity of radioactive sulfate in the gastric contents decreases in a significant manner (total amount: 3.7% of the control value,  $p < 0.01$ ) as well as its concentration per ml ( $p < 0.01$ ). Restraint provokes a significant increase ( $p < 0.05$ ) of the total amount and of the concentration of non-dialysable radioactive sulfate in the pure gastric juice. The decrease observed in the wall (total amount: 87.5% of the control value) is not significant.

3. In rats of group II, bearing a ligature between the rumen /116 and the glandular stomach, restraint causes a significant decrease of radioactive sulfate in the saliva (total amount: 12.4% of the control value,  $p < 0.005$ . Concentration per ml: 14.2% of the control value,  $p < 0.02$ ) (Table II).

4. When saliva is collected in an isolated manner by esophageal catheter and balloon (group III), restraint provokes a significant /117 decrease of the salivary radioactive sulfate (total amount: 28.1% of the control value,  $p < 0.01$ . Concentration: 39.3% of the control value,  $p < 0.01$ ) (Table II).

Discussion. - Restraint for 6 hours provokes in rats with pyloric ligation a marked change in the amount of non-dialysable radioactive sulfate in the combination "gastric wall + gastric contents", which then reaches 54% of the control value. This change is essentially linked to the decrease of sulfate in the gastric contents (total amount: 33% of the control value).

The degree of contamination of the gastric juice by salivary radioactive sulfate is demonstrated by the drop-off of the radio-

Experimental series	Controls		Restraint	
	pyl lig.	Pyl lig/Cerv. Esoph. lig.	pyl lig.	Pyl lig/Cerv. Esoph. lig.
No. of rats	16	16	13	15
Dry weight (mg)	268.7	304.9	254.9	263.9
Gastric wall				
Total amount (dpm 6 hr)	349 000 (24 200)	344 000 (27 300)	288 000 (29 400)	301 000 (18 400)
Concentration (dpm/mg)	1 263 (96.5)	1 263 (130.5)	1 193 (124.7)	1 099 (70.4)
Gastric contents				
Volume (ml)	7.03	1.58	3.18	2.08
Total amount (dpm 6 hr)	615 000 (176 500)	23 000 (3 300)	209 000 (27 500)	59 000 (8 800)
Concentration (dpm/ml)	78 300 (15 300)	19 100 (2 400)	71 200 (10 300)	34 500 (5 600)
Total				
Total amount (dpm 6 hr)	911 000 (184 300)	362 600 (28 500)	497 400 (46 800)	360 500 (25 800)

TABLE I. EFFECT OF RESTRAINT ON RADIOACTIVE SULFATE IN THE STOMACH CONTENTS AND WALL OF RATS WITH PYLORIC LIGATION, WHETHER SUBJECTED TO AN ESOPHAGEAL LIGATION OR NOT. IN RATS WITH PYLORIC LIGATION, RESTRAINT CAUSES A DECREASE OF RADIOACTIVE SULFATE IN THE GASTRIC JUICE. AFTER ESOPHAGEAL LIGATION, INTRAGASTRIC RADIOACTIVE SULFATE FALLS AND THE EFFECT OF RESTRAINT IS REVERSED.

Groups of experiments		Group II		Group III	
Experimental conditions		Mediogastric Ligature		Esophageal Catheter	
		Controls 8 rats	Restraint 8 rats	Controls 15 rats	Restraint 11 rats
Volume (ml)		3.1 (1.1)	1.8 (0.4)	2.2 (0.2)	1.7 (0.3)
35 S	Dpm/6 hr	542 200 (191 700)	67 300 (15 000)	1 190 000 (173 800)	334 200 (107 000)
	Dpm/ml	216 600 (71 900)	30 800 (7 300)	557 800 (74 600)	219 000 (63 600)

TABLE II. EFFECT OF RESTRAINT ON THE RADIOACTIVE SULFATE IN THE SALIVA COLLECTED PROXIMAL TO A MEDIOGASTRIC LIGATURE OR BY AN ESOPHAGEAL CATHETER. WHATEVER PROTOCOL IS UTILIZED TO COLLECT THE SALIVA, RESTRAINT PROVOKES A SIGNIFICANT DECREASE OF THE TOTAL AMOUNT AND OF THE SALIVARY CONCENTRATION OF RADIOACTIVE SULFATE WITHOUT ANY SIGNIFICANT CHANGE IN VOLUME.

activity of the gastric contents after esophageal ligation (group I.b). This is in accord with the decrease of hexosamines in the gastric contents observed after esophageal ligation [5]. The salivary secretion collected in the pure state (groups II and III) contains a considerable quantity of radioactive sulfate, the values being more elevated in group III because of stimulation of salivary secretion by the intraesophageal catheter. Restraint provokes a marked decrease in the radioactive sulfate excreted in the saliva (groups II and III). This change is in part linked to the immobilization of the animals, incapable of licking themselves. However, a decrease associated with synthesis of sulfated glycoproteins in the saliva can be suggested because the change in the concentration of the radioactive sulfate is more marked than that in volume.

Pure gastric secretion is collected in rats with esophageal ligation (group I.b). The quantity of radioactive sulfate present in the gastric juice is very small. It is necessary, however, to take

into account a possible inhibitory effect of the esophageal ligation on the gastric glandular mucosa [6]. The size of the change provoked by restraint in these conditions is modest, but favors an increased excretion of glycoproteins.

In rats with pyloric ligation, the amount of non-dialysable radioactive sulfate in the gastric wall falls during restraint (total amount: 82% of the control value), this change is not significant. A greater change has previously been reported [1,2] but the experimental protocol was different: analysis was carried out electively on the radioactive sulfate in the glandular mucosa separated from the gastric muscular tunic, while our study was carried out on the entirety of the glandular stomach wall.

In conclusion, restraint provokes a marked decrease in non-dialysable radioactive sulfate incorporated in the saliva. This effect is the principal factor in the change in gastric contents of rats with pyloric ligation subjected to restraint. When the salivary factor no longer plays a role, an increase of excretion of radioactive sulfate into the gastric vent is observed under restraint with no decrease in the sum of "parietal radioactive sulfate + radioactive sulfate in the secretion".

Summary. Study of non-dialysable radioactive sulfate in the gastric wall and contents in rats should take into account gastric contamination by salivary sulfated glycoproteins. During six hour experiments, restraint provokes a significant decrease in salivary radioactive sulfate. This, in turn, is responsible for the decrease of sulfate in the gastric contents observed under these conditions in rats with pyloric ligation. Esophageal ligation associated with this prevents passage of saliva and lowers the amount of radioactive sulfate in the gastric juice. Restraint causes then an increase in the amount of sulfate in the gastric juice, the value observed being very much lower than that of rats with a free esophagus. At the level of the gastric wall, the change observed during restraint does not reach a significant threshold within the framework of this study.

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