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Influence of the ABO blood group and salivary ABH secretor status on the cell-removing effect of aspirin on human gastric mucosa

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EDITORIAL COMMENT The association of duodenal ulcer with blood group O and salivary ABH non-secretion, and the apparent increased likelihood of bleeding in patients of blood group O with duodenal ulcer, are not understood. A new approach to the problem is provided by this carefully executed investigation which demonstrates an interaction between blood group and secretor status, and cell loss from gastric mucosa washed with an aspirin solution.

It has been realized since the work of Patzelt (1882) and Bizzozero (1892) that the zones of mitotic activity in the gastric mucosa and in the crypts of Lieberkuhn of the small bowel were zones of regeneration from which the newly-formed cells could move up or down to replace those which are worn out or damaged. Various techniques have given estimates of one to six days as the time required to produce a new covering of cells for the gastric mucosa in animals (Stevens and Leblond, 1953; Messier and Leblond, 1960; Creamer, Shorter, and Bamforth, 1961; Weirnick, Shorter, and Creamer, 1962). In man Lipkin, Sherlock, and Bell (1963) utilized the injection of tritiated thymidine followed by peroral biopsy of gastric mucosa. Labelled cells took 48 to 96 hours to reach the surface of the gastric pits from their site of origin.

Using the technique of mitotic counts in operation specimens, Teir and Räsänen (1961) showed that the non-pathological mucosa in patients with gastric ulcer and gastric carcinoma has a more rapid turnover of cells than in those with duodenal ulcer.

Associations are known between ABO blood groups and gastrointestinal disorders, and also the genetically determined character of salivary ABH non-secretion is associated with duodenal ulcer (Roberts, 1957).

It would therefore be of interest to know what effect, if any, do the ABO blood group and salivary ABH secretor characters have on the cell kinetics of the gastrointestinal tract.

A useful technique to investigate this problem has been developed by Croft (1963) which allows a quantitative assessment to be made of the numbers of cells shed from the human gastric mucosa. This method circumvents the difficulties of cell counting by making a homogenate of the shed cells and estimating the DNA (desoxyribonucleic acid). All cells contain the same amount of DNA and hence estimating this substance gives an expression for the numbers of cells.

Aspirin (acetylsalicylic acid) can be shown to remove cells at an enhanced rate from the gastric mucosa (Croft, 1963). It seemed to be of interest to determine whether ABO blood group and secretor status influenced the cell-removing effect of aspirin on the gastric mucosa.

MATERIALS

VOLUNTEER SUBJECTS The procedure was not practicable on completely healthy subjects, therefore volunteer patients were used from the medical, surgical, and psychological medicine wards of a large general hospital. Subjects were all ambulant and eating a full hospital diet. The experiment was done to a factorial design, so the first 20 male O secretor, first 20 male O non-secretor, etc., patients were tested until 20 subjects had been tested in all eight phenotypical groups. Subjects of blood groups B and AB, with pregnancy or peptic ulcers, on steroids or salicylates, with purulent sputum, and those who had received surgery, were not employed. A total of 623 suitable patients were 'screened' with blood and saliva tests until the experimental design of 160 subjects was completed.

SALIVA Saliva was collected by having the subject spit into a test-tube; it was boiled for 10 minutes within two hours of collection and then stored at −20°C.

ANTISERA A saline extract of *Ulex europaeus* seeds prepared by the method of Boyd and Shapleigh (1954) was used as an anti-H agglutinin. Anti-A and anti-B were of the non-immune type from human sera. The same standardized solutions of the agglutinins were used throughout.

WORKING DNA STANDARD This was prepared from purified calf thymus DNA as detailed by Croft (1963).

METHODS

SALIVA The saliva was thawed at room temperature after removal from storage at −20°C. (Even though the saliva had been centrifuged previously a little gelatinous precipitate forms after freezing.) The clear supernatant was used for agglutination-inhibition tests.

CLASSIFICATION INTO SALIVARY ABH SECRETOR PHENOTYPE All salivas were tested for H, A, and B activity by means of an agglutination-inhibition technique (Race and Sanger, 1962) with 0·145M sodium chloride as the diluent. Standard salivas from known blood group O, A, and B secretors and from non-secretors were included in each day's estimations. One-half, one-fourth, and one-eighth dilutions of saliva were tested for ability to inhibit agglutination.

RED CELL GROUPING This was carried out in the usual manner by means of a tube technique (Lawler and Lawler, 1957).

PROCEDURE ON THE INDIVIDUAL SUBJECT The procedure was carried out as detailed by Croft (1963) so that each subject yielded four stomach washings, namely, resting, control, aspirin I, and aspirin II.

In essentials the procedure is as follows. Fasting subjects had their stomachs evacuated of residual juice. Physiological saline was introduced into the stomach and washed out with further saline to make a total of 500 ml. This was termed the 'resting' wash. Then saline solution was allowed to remain in the stomach for five minutes and was subsequently washed out with saline to yield a 'control' wash. Thereafter an aspirin solution was introduced into the stomach for five minutes and subsequently washed out with saline. This was called the 'aspirin I' wash. Another similar procedure wherein the aspirin solution remained in the stomach for 10 minutes yielded the 'aspirin II' wash.

PROCEDURE ON THE GASTRIC WASHINGS The gastric washings were processed in the way described by Croft (1963), with the slight modifications noted below. Protein precipitation and homogenization were performed on each specimen and then two duplicate aliquots were taken for DNA extraction. Preliminary experiments had shown that a second DNA extraction step ensured that the total extraction exceeded 95%. Therefore a second extraction was performed by adding 2 ml. of 5% trichloroacetic acid to each deposit and after again mixing to an even suspension the contents were heated at 90°C. for 15 minutes. After cooling and centrifuging the supernatant solution was decanted into the test-tube containing the first extraction from that particular aliquot.

There is a slowly progressive colour development when DNA is estimated by the diphenylamine reaction. This flattens out after the tubes have stood for 24 hours in the dark, and is then stable. Therefore all tubes in this experiment were read after standing 36 to 48 hours under these conditions.

The agreement between duplicates was very close. To quantitate the agreement the duplicate optical density readings on the aspirin II wash of the first 40 subjects investigated have been used for computation. The variability within individuals, *i.e.*, between duplicates, is 0·172% of the total variability of the 80 readings.

There was no significant interference with the DNA readings by aspirin solution; also sialic acid added at a

TABLE I

MEANS AND THEIR STANDARD ERRORS OF (LOG_{10} + 1) TRANSFORMATION OF THE DNA CONCENTRATIONS IN GASTRIC WASHINGS

Nature of Washings Analysed	*ABO Blood Groups and Salivary ABH Secretor Phenotype*	*Sex*	
		Male	*Female*
Resting	O Secretor	1·097 ± 0·093	0·997 ± 0·082
	O Non-secretor	1·242 ± 0·074	1·091 ± 0·092
	A Secretor	1·154 ± 0·062	1·051 ± 0·063
	A Non-secretor	1·216 ± 0·081	1·197 ± 0·069
Control	O Secretor	0·965 ± 0·084	0·826 ± 0·075
	O Non-secretor	0·919 ± 0·089	0·817 ± 0·056
	A Secretor	0·852 ± 0·078	0·779 ± 0·044
	A Non-secretor	0·880 ± 0·072	0·996 ± 0·063
Aspirin I	O Secretor	1·106 ± 0·065	0·885 ± 0·074
	O Non-secretor	0·895 ± 0·063	0·857 ± 0·051
	A Secretor	0·857 ± 0·072	0·878 ± 0·057
	A Non-secretor	0·930 ± 0·063	1·014 ± 0·068
Aspirin II	O Secretor	1·151 ± 0·059	0·973 ± 0·055
	O Non-secretor	1·124 ± 0·077	0·884 ± 0·071
	A Secretor	1·048 ± 0·079	0·982 ± 0·044
	A Non-secretor	1·149 ± 0·050	1·205 ± 0·073

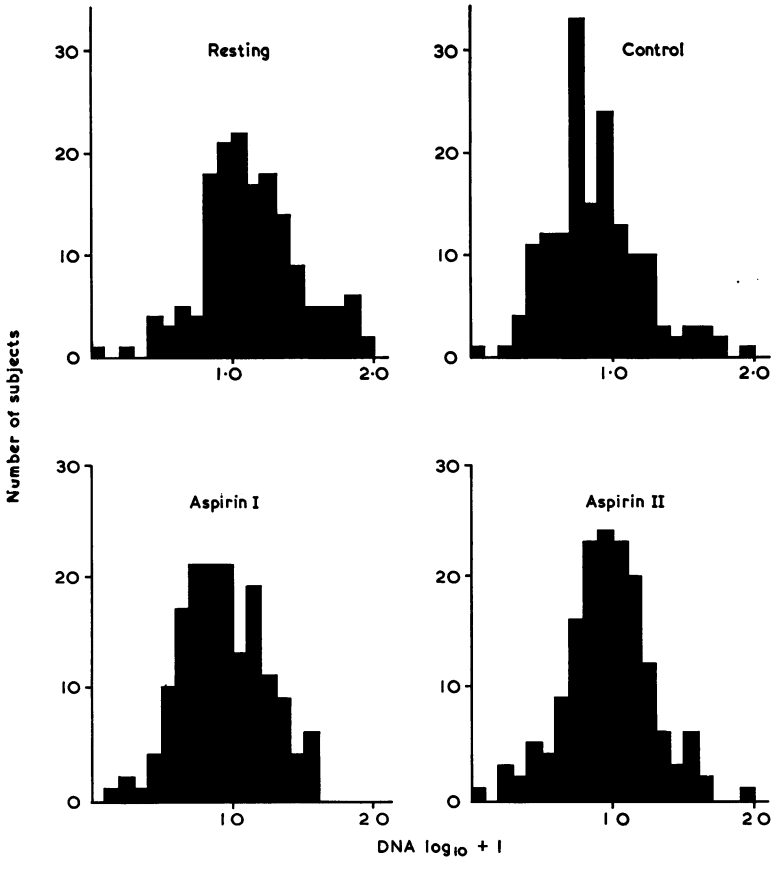

FIG. 1. *The DNA values for the resting, control, aspirin I, and aspirin II washes in log_{10} form.*

concentration of 1 mg. per millilitre to DNA at a concentration of 0·42 μg. atom P per millilitre showed no significant interference.

STATISTICAL METHODS These were standard (Snedecor, 1956).

RESULTS

The DNA values for the resting, control, aspirin I, and aspirin II washes are shown in Fig. 1 in log_{10} form and are normal in distribution.

The means with their standard errors of the log_{10} values for the eight phenotypic groups are shown in Table I. Aspirin values are larger than control values, especially aspirin II in males.

Both the resting and control values were correlated with the aspirin values, to a similar degree. The resting values were, however, high as compared with the control values, suggesting that these resting samples contained DNA which had been lying in the stomach for some time and might contain contributions of DNA of extragastric origin. Therefore the resting values were not analysed further.

FACTORIAL ANALYSIS The aspirin values were added together and then the log_{10} of the sum taken. Following this 1·0000 has been added to all values in order to eliminate 'bar 1' values for ease of computation. This leads to the population shown in Fig. 2 which approximates to a normal distribution curve, as do the deviations from the phenotypical means. A factorial analysis has been performed (Table II). Three significant effects contributing to the variance have been found, and they can be followed in the mean values listed in Table III. They are as follows:

1 Females have on the whole lower values than males. 2 In subjects, both male and female, of blood group O the non-secretors have lower values than the secretors, whilst the converse is true in subjects of blood group A. 3 In subjects of blood group O (when secretors and non-secretors are added together), males have higher values than females, whilst the converse is true in subjects of blood group A.

TABLE II

FACTORIAL ANALYSIS OF DNA VALUES FOR ASPIRIN DATA IN THE TRANSFORMATION $(Log_{10}(Aspirin\ I + Aspirin\ II)) + 1$

Item	*Degrees of Freedom*	*Sum of Squares*	*Mean Squares*	*F*	*p*
Blood group (BG)	1	0·0751	0·0751	1·33	NS
Secretor status (SR)	1	0·0449	0·0449	< 1	NS
Sex (SX)	1	0·2308	0·2308	4·10	<0·05
Interaction BG × SR	1	0·5283	0·5283	9·38	<0·01
BG × SX	1	0·4019	0·4019	7·14	<0·01
SX × SR	1	0·1021	0·1021	1·81	NS
BG × SR × SX	1	0·0321	0·0321	<1	NS
Residual	152	8·5604	0·0563	—	—
Total	159				

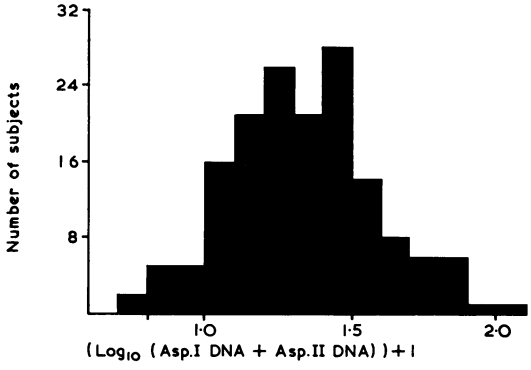

FIG. 2. $(log_{10}(Asp.I\ DNA + Asp.II\ DNA)) + 1$.

The first effect is one of a main factor, whereas the last two are interactions. The means for the control values show a similar pattern (Table I), but the effects do not reach significance on factorial analysis. The combined aspirin data are derived from pooling two sets of observations against one set for the control data and the differences between groups may be greater after aspirin just because the results at this stage have less random error.

DISTRIBUTION OF AGE AND WEIGHT IN EXPERIMENTAL SUBJECTS The data for age and weight have been subjected to factorial analysis. There was a slight but significant difference in ages, the men being older than the women.

TABLE III

MEANS AND THEIR STANDARD ERRORS FOR DNA VALUES $(Log_{10}(Aspirin\ I + Aspirin\ II)) + 1$

ABO Blood Group and Salivary ABH Secretor Phenotype	*Sex*	
	Male	*Female*
O Secretor	1·450 ± 0·238	1·252 ± 0·243
O Non-secretor	1·347 ± 0·250	1·193 ± 0·221
A Secretor	1·307 ± 0·279	1·252 ± 0·183
A Non-secretor	1·376 ± 0·204	1·480 ± 0·219

The data for weight showed powerful main factor contributions of sex and secretor status to the variability between the phenotypical classes (Tables IV and V). The men were heavier than the women, and the non-secretors heavier than secretors.

TABLE IV

MEANS AND THEIR STANDARD ERRORS FOR WEIGHT (kg.)

ABO Blood Group and Salivary ABH Secretor Phenotype	*Sex*	
	Male	*Female*
O Secretor	66·05 ± 2·48	50·20 ± 2·01
O Non-secretor	74·64 ± 3·01	56·30 ± 1·93
A Secretor	67·52 ± 3·06	58·91 ± 2·23
A Non-secretor	72·75 ± 3·51	60·27 ± 2·85

TABLE V

FACTORIAL ANALYSIS OF WEIGHT (kg.)

Item	*Degrees of Freedom*	*Sum of Squares*	*Mean Squares*	*F*	*p*
Blood group (BG)	1	377	377	2·62	<0·05
Secretor status (SR)	1	1,131	1,131	7·85	<0·01
Sex (SX)	1	7,638	7,638	53·04	<0·01
Interaction BG × SR	1	163	163	1·13	NS
BG × SX	1	428	428	2·97	NS
SX × SR	1	101	101	0·70	NS
BG × SR × SX	1	6	6	0·04	NS
Residual	152	21,951	144	—	—
Total	159				

TABLE VI

PARTIAL REGRESSIONS CALCULATED
($n = 160$)

x_1	x_2	y	b_1	b_2	$se(b_1)$	$se(b_2)$	$\frac{b_1}{se(b_1)}$ and p	$\frac{b_2}{se(b_2)}$ and p
Age (yr.)	Weight (kg.)	Aspirin I + aspirin II[2] + 0·000137	+ 0·002047	0·001573	0·001804	0·0873	1·1346
							>0·10	>0·10
Age (yr.)	Weight (kg.)	Control[1]	− 0·002190	+ 0·000651	0·001573	0·001804	1·3923	0·3609
							>0·10	>0·10

[1]Control = ($\log_{10}$ control DNA) + 1
[2]Aspirin I + aspirin II = ($\log_{10}$ (Aspirin I DNA + Aspirin II DNA)) + 1
b = regression line (slope)
$se(b)$ = standard error of regression line (slope)

RELATION OF AGE AND WEIGHT TO DNA VALUES The relationship between these three variables was studied by the calculation of partial regressions. There is no significant regression of DNA values upon either age or weight (Table VI). Therefore the significant effects on ages and weights are not responsible for those shown in Table II.

DISCUSSION

Possible sources of error in the production of the results must be considered. Gastric emptying is so capricious that gastric contents may be lost through the pylorus at any time during the test. It is not possible to make any correction for this loss, since it may occur at the beginning or at the end of a washout and the amount of cellular material would be correspondingly great or small. There is no evidence, however, to suggest that emptying is associated with blood group and secretor status, and loss through emptying should be randomly distributed through the population of 160 subjects studied.

Emotional factors may well be important, although all steps were taken to minimize them. Adrenalin is a most potent inhibitor of gastric mucosal cell turnover (Bullough, 1962), but again this factor seems unlikely to be associated with any of the factors tested by the experimental design.

The significant effects on factorial analysis of the aspirin data could have been the result of unconscious bias, for example, by selecting patients whose diseases have not so far been investigated for blood group or secretor association. There was no predominant disease in any of the eight phenotypical groups, and no phenotypical group was selected to an unusual degree from a particular department of the hospital. Only five of the 160 patients turned out to have carcinoma (two breast, one bronchus, one penis, one colon).

It seems likely that aspirin, being unionized and fat soluble in an acid environment (Davenport, 1964) is able to penetrate into and between the mucosal cells, so damaging the cells and their inter-cellular cement so that large numbers fall off into the gastric lumen.

In the present experiments many of the gastric washouts following aspirin contained opaque mucus which settled out on standing. Microscopic examination showed this material to contain masses of cell nuclei, sometimes together with sheets of intact epithelial cells. The DNA estimated in the present work gave an index of this process, which seems similar to the 'protective' opaque cellular mucus described by Hollander, Stein, and Lauber (1946).

The males tended to lose more mucosal cells into the gastric lumen than females. The probable explanation for this phenomenon is that the males had the larger stomachs. The weight of abdominal viscera is correlated to body weight (in rats, Hatai, 1918) and in the present work the males were heavier than the females. Cox (1952) showed that mean gastric mucosal area was 850 sq. cm. in men and 783 sq. cm. in women. It also seems possible that hormones, such as oestradiol and testosterone, may influence the cell-shedding properties of the mucosa in response to aspirin.

'Interaction means different things to different people. To the chemist, it is much the same as reaction. The physiologist working with hormones thinks of mutual stimulation among the glandular activities. The agronomist may be interested in the effect of one plant nutrient as conditioned by the availability of a second. To the statistician interaction is measured by the 'failure of effects to be additive' (Snedecor, 1956). Statistically, it is unusual to find a significant interaction without demonstrating significant effects of the factors involved in the interaction when these are considered singly.

The effect of the interaction of blood group and secretion on the response of the gastric mucosa to aspirin cannot be explained in the light of present day knowledge. It seems possible that the gastric mucosal cell turnover and mucous cell maturation may be relevant factors. These processes may be related to

the ABO blood groups and secretor status. Teir and Räsänen (1961) find a more rapid mucosal cell turnover rate in the non-neoplastic gastric mucosa of gastric carcinoma subjects than in the mucosa of duodenal ulcer subjects. The former disease is associated with blood group A and the latter with blood group O. It can be speculated that the mucosa which withstands injury by aspirin most efficiently is determined by the combination of secretion with one blood group and non-secretion with the other.

The explanation for the significant interaction between blood group and sex is also obscure; but if these results are confirmed by further work, this phenomenon may also be connected with the dynamics of the mucosal cell population, turnover and maturation.

It would seem that aspirin merely enhances the pattern of shedding which occurs in the mucosa in the control wash simulating more 'physiological' conditions.

The very surprising finding that the non-secretors were heavier than the secretors was quite unexpected. No explanation for this observation is known. Like the gastric cell shedding results it should be confirmed by further observations.

SUMMARY

Cell shedding from the gastric mucosa has been studied by measuring the DNA content of gastric washings. Control saline washings and washings obtained following the intragastric instillation of aspirin solutions have been analysed.

One hundred and sixty experimental subjects have been studied to a factorial design to assess the influence of three factors on gastric cell shedding, namely, sex, ABO blood group, and salivary ABH secretor status.

The results on the aspirin washings show a significant effect of (1) sex as a main factor; (2) the interaction of blood group and secretor status; and (3) the interaction of sex and blood group.

An unexpected additional significant finding was that non-secretors were heavier than secretors.

These findings are discussed.

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