Biochemical Pharmacology, Vol. 27, pp. 2775–2780.

THE EFFECT OF DRUGS AND SECRETAGOGUES ON THE BIOSYNTHESIS OF GASTRIC MUCINS

DEIRDRE WALDRON-EDWARD*, CATHERINE DECAËNS†, ALEXANDRE ROBERT†, JEAN-PIERRE BADER‡, LADISLAS ROBERT† and JACQUELINE LABAT-ROBERT†

*Department of Surgery, Donner Building, McGill University, 740 Mc Gregor, Montreal, Quebec, H3A 1 4A, Canada

†Laboratoire de Biochimie du Tissu Conjonctif, Faculté de Médicine, Université Paris XII, 94000 Créteil, France

and ‡Service de Gastro-Entérologie, Hôpital Henri-Mondor, Université Paris XII, 94000 Créteil, France

(*Received* 28 *September* 1977; *accepted* 9 *December* 1977)

Abstract—Gastric mucosal glycoproteins were separated into three well-defined fractions: secreted mucin *in vivo*, adherent to cells, Fraction I; soluble mucin secreted during incubation *in vitro*, Fraction II; and a third, Fraction III released solely by pronase digestion (cell bound or intracellular mucin). ^{14}C-U-D-glucose and $^{35}SO_4$ incorporation into glycoprotein fractions II and III was studied during a 4-hr incubation period *in vitro*, of mucosal scrapings, freed from Fraction I, from rats treated *in vivo*, with secretin, histamine, aspirin and atropine. The effect of *in vitro* added puromycin and actinomycin was also studied. Secretin decreased and histamine increased the total amount of protein and glycoprotein in Fraction II. Histamine inhibited incorporation of glucose into this fraction, to a moderate extent. Secretin had no effect on incorporation. Per oral aspirin, administered for 15 min, decreased levels of secreted mucin Fraction I, and strongly inhibited glucose incorporation in Fraction III. Parenterally administered aspirin and atropine decreased mucin secretion *in vivo* (Fraction I) but activated glucose incorporation slightly in Fraction II and strongly in Fraction III. Puromycin and actinomycin added *in vitro* to the mucosal scrapings inhibited strongly glucose incorporation in Fraction III, but did not inhibit incorporation in Fraction II. This indicates the existence of two separate phases of mucin biosynthesis and secretion, both necessitating glucose incorporation, but only the first one being sensitive to puromycin and actinomycin. $^{35}SO_4$ incorporation was strongly inhibited by all drugs studied. Sulphation of mucins appears to be much more sensitive to drug action than mucin biosynthesis itself. The above results indicate that the described system is suitable for the study *in vitro* of the time sequence and mechanisms of drug action.

Mucin glycoproteins comprise almost 50 per cent of the dry wt of the mucosal tissue of the gastric antrum, although somewhat less in the main body of the stomach. The estimation of the rate at which this major macromolecule is biosynthesized, as well as the composition of both secreted and secretable mucin, is of great importance for the evaluation of the normal or pathological functioning of the gastric mucosa. It was shown recently that pig [1], rat [2, 3] and human [4–6], gastric mucosal scrapings or biopsies actively incorporate radioactive precursors such as ^{14}C-U-D-glucose, ^{14}C-L-1-fucose or [G-^{3}H]-threonine, [G-^{3}H]serine and $Na_2{}^{35}SO_4$ in both secretable mucin and into cell bound glycoproteins. ^{14}C-glucose was shown to be incorporated into the peptide [7] and carbohydrate fractions [1, 7] of these glycoproteins; incorporation was linear during a 4-hr incubation period [3]. This model appeared therefore particularly suitable for the systematic exploration of the effect of drugs or hormones on the rate or quality (carbohydrate–protein ratio) of mucin synthesis and secretion. We propose to describe here the results obtained with some effectors and drugs, such as secretin, histamine, atropine and aspirin, administered *in vivo*. These drugs were compared in action to that of puromycin and actinomycin, added *in vitro*.

Previous experiments by Kent and Allen [4, 5] have shown that aspirin and sodium salicylate, added to cell suspension *in vitro*, inhibit the incorporation of ^{14}C-glucose into whole, unfractioned glycoprotein moiety of gastric and colonic mucosal scrapings from humans and sheep, respectively. The other drugs mentioned were not investigated in similar systems, to our knowledge.

The glycoproteins of the gastric mucosa were separated into three distinct fractions, as previously described [2, 3] that is (1) the film of secreted mucin, closely adherent to the cell walls solubilized by washing (fraction I); (2) the mucin-like glycoprotein synthetized and secreted during incubation (fraction II), and finally, a fraction which is rendered soluble only by digestion with pronase (fraction III or cell-bound fraction).

The effect of the above drugs on the incorporation of ^{14}C-glucose and $^{35}SO_4$ in these mucin-fractions *in vitro* was studied in order to distinguish between their actions on the biosynthesis of epithelial glycoproteins and their secretion *in vivo*.

MATERIALS AND METHODS

Administration of the drugs. Wistar rats were fasted overnight. Groups of five rats were treated in the following ways at the dosage denoted on each Table: histamine (Betazole) was injected intramuscularly, 1 hr before decapitation. Secretin in saline was injected into each rat, 1 hr before sacrifice.

Atropine, was injected, 5 min before sacrifice. Aspirin (as aspirin–lysine) dissolved in saline, 500 mg per rat, was injected i.p. This amount of aspirin caused animal death within 5 min in a few cases. In a second series, aspirin-lysine was reduced to 50 mg: all animals survived for 60 min after injection. An aqueous suspension of oral aspirin was administered by a nasogastric tube to lightly anaesthetized rats; the animals were decapitated after 15 min. All experiments were duplicated.

Preparation of gastric mucosal scrapings. The stomachs are excised after sacrifice and transferred to a cold room at 4°, opened and rinsed rapidly in ice cold saline, then carefully scraped. The scrapings from five rats are pooled, divided into two, weighed and suspended in ice-cold Hank's medium, with sharp, vigorous agitation.

Three washings are necessary to allow adherent mucin to swell and disintegrate. The saline washings of the mucosal scraping contain preformed mucin *in vivo* adherent to the gastric mucosa. This mucin dispersion is termed Fraction I and analysed for its protein and carbohydrate contents.

The washed cell suspension is centrifuged at low speed, 500 *g* at +4° for 5 min, resuspended in Hank's or Eagle's MEM medium containing 25 μM ATP (5 ml for the scrapings from two rat stomachs), evenly dispersed by swirling transferred to 25 ml cultured flasks, the radioactive tracer is added (20 μCi ^{14}C-U-glucose, specific activity 250 mCi/mM, CEA, Saclay; or 10 μCi $^{35}SO_4$, spec. act. 3.6 mCi/mM, CEA, Saclay) and the flasks are incubated in a shaking water bath at 37° for 4–5 hr. At the end of the incubation "cold" glucose or sulphate is added (1% final concentration), the suspension is centrifuged at +4° and the insoluble residue rinsed twice with 5 ml cold 0.9% NaCl. The combined supernates are exhaustively dialysed against running tap water and distilled water and represent "fraction 2". They contain radioactive glycoproteins (mucins) released by the cell suspension during incubation.

The washed cell residue is then incubated in 2 ml Tris–HCL-buffer 0.1 M pH 8, 0, pronase Calbiochem, 80 mg/100 ml, is added and the suspension is incubated overnight at 37°. This treatment is repeated once. After exhaustive digestion, the suspension is centrifuged at 5000 *g* for 15 min at +4° and dialysed against running tap water and distilled water for 48 hr. This pronase-extract is termed "fraction 3" and contains intracellular mucins.

The three mucosal fractions obtained in this way were analyzed for fucose by the cystein-sulfuric acid procedure of Dische [8], for hexoses [9], using the orcinol method, for hexuronic acids [10] using the carbazol method of Dische and for proteins [11] by Lowry's procedure.

The precision and specificity of these colorimetric methods have been exhaustively studied by Dische [8, 12] Montreuil and Scheppler [13], Montreuil and Spik [9]. The interference of proteins in the orcinol estimation of hexoses (essentially galactose) is negligible at 510 nm. The interference of hexoses in the fucose estimation is negligible if the absorbance measurements are done at 396 and 425 nm. The interference of fucose in the orcinol estimation of hexoses is corrected for by using adequate standards.

Aliquots were added to scintillation fluid and counted in an Intertechnique Counter, correcting for counting efficiency, and decay in the case of $^{35}SO_4$. Water content of mucosal scrapings was determined by drying samples to constant weight in vacuo, over P_2O_5.

All three fractions obtained by the above procedure are rich in hexoses and in fucose [3]. Fraction I contains preformed mucin *in vivo* and the variations of its composition reflect the action *in vivo* of the administered drugs. Modification in the composition and/or in the radioactivity of Fraction II (released mucins *in vitro*) and of Fraction III (intracellular mucins) reflect the action of drugs on the mucin synthesising cells themselves. Most radioactivity (> 90%) in Fraction III is non-dialysable showing that it is present in the pronase resistant glycopeptide core. Fraction III-mucins are considered as in the process of biosynthesis and not yet excreted. Differences in drug action were noticed on mucin biosynthesis at the level of Fraction II and Fraction III suggesting that different mechanisms are involved (see Results). More details on these methods are given elsewhere [2, 3, 14, 15].

RESULTS

Action of drugs on mucin composition. As shown in Fig. 1, injection of neither secretin nor histamine had any appreciable effect on the amount of bound neutral carbohydrate (hexose + fucose to protein ratio) in Fraction I, containing secreted mucins *in vitro*. Aspirin, on the other hand, whether given orally or parenterally, markedly reduced the carbohydrate–protein ratio in this Fraction. Atro-

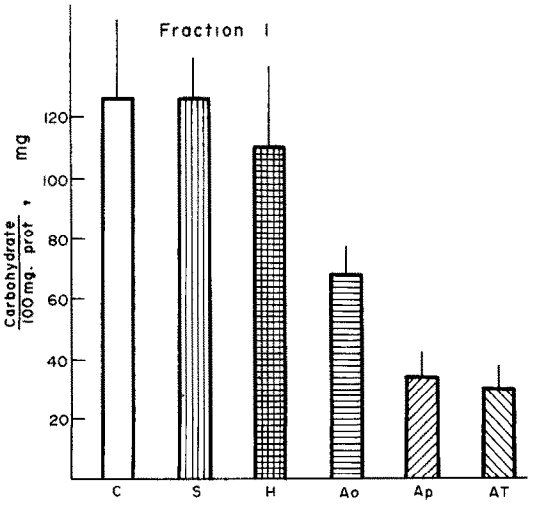

Fig. 1. Carbohydrate (galactose plus fucose) to protein ratio of Fraction I. This was obtained by pooling three slow washings (total period, 1 hr), with vigorous agitation (4 × 30 sec) each time, in order to allow secreted gel mucin, adherent to mucosal cells, to swell and disintegrate. C = control; S = secretin and H = histamine injections; Ao = oral aspirin; Ap = parental aspirin; AT = atropine injections. Average of four to five experiments ± S.E.M.

Table 1. Carbohydrate (hexose + fucose) and protein in washed mucosal scrapings from rats treated *in vivo* with different effectors, compared with normal mucosa, treated *in vitro*, with inhibitors of protein and RNA synthesis

Carbohydrate (fucose + hexose) and protein were assayed as described in the text. Figures are the average of n experiments ± S.D., each assay being carried out in duplicate. (°)$P \leqslant 0.005$; (°°)$P \leqslant 0.025$. Average wt of rats; (a) 500 g (b) 250–300 g

Effector	n	Dose	Route	time (min)	Fraction II; released mucin Gal + Fuc μg/100 mg	Protein fresh tissue	Gal + Fuc% prot	Fraction III; cell bound glycoprotein Gal + Fuc μg/100 mg	Protein fresh tissue	Total Gal + Fuc II + III μg/100 mg fresh tissue
(a) Control	4				344 ± 124	538 ± 42	61 ± 7	232 ± 27	254 ± 21	576
Secretin	4	2 i.u./kg	i.p.	60	263 ± 120	°°401 ± 76	65 ± 5	236 ± 47	201 ± 27	499
Aspirin	4	200 mg/kg	per os.	15	521 ± 148	509 ± 69	92 ± 12	350 ± 58	364 ± 42	871
Aspirin	2	200 mg/kg	i.p.	60	317	440	79	273	411	690
Histamine	2	100 μg/kg	i.m.	60	357	695	54	247	375	604
Atropine	2	2 mg/kg	i.p.	5	410	683	60	414	297	824
(b) Control	4				410	983	40	357	433	767
Aspirin	2	200 mg/kg	i.p.	60	497	1335	37	372	484	869
Histamine	2	100 μg/kg	i.m.	60	611	1460	42	280	525	891
Atropine	2	2 mg/kg	i.p.	5	551	1029	53	395	575	1126
(b) Control	2				373	994	43	350	430	723
		in vitro								
Puromycin	2	250 μg/100 mg fresh tissue			540	1070	54	309	430	849
Actinomycin	1	12.5 μg/100 mg fresh tissue			413	1204	34	397	460	910

pine had an effect very similar to the aspirin treatments.

The total non-dialysable bound carbohydrate and protein obtained from Fraction II and Fraction III, from untreated rats, decreased somewhat with the size (age) of the rats (Table 1). Thus, medium sized rats (250–300 g) yielded 3.62 ± 0.3 mg hexose + fucose per 100 mg dried tissue, whereas mucosa from rats weighing 500 g contained only 2.88 ± 0.4 mg/100 mg. Smaller rats (150 g) yielded even higher figures (1100 to 1300 μg carbohydrate per 100 mg fresh mucosa (Waldron-Edward and Greenberg, unpublished results).

Table 1 shows the composition (Gal + Fuc and proteins) of Fractions II and III from control and treated animals. Carbohydrate–protein ratios are also shown for Fraction II but not for Fraction III because of the proteolytic treatment this fraction has undergone.

Although the glycoprotein and protein content of mucin released during incubation, Fraction II, and of cell-bound glycoproteins, Fraction III, from control and treated animals varied, the carbohydrate–protein ratio in each series of experiments remained relatively constant. Secretin, however, significantly decreased both protein and carbohydrate content in Fraction II. Atropine, aspirin and histamine on the other hand increased the total glycan content within the washed mucosal mass. This increase may be attributed to an inhibition of secretion of preformed mucin corresponding probably with the decrease observed in Fraction I (see Fig. 1).

The effect of puromycin and actinomycin added *in vitro*, is also shown on Table 1. Neither additive appeared significantly to modify the overall carbohydrate content of either Fractions II or III, although more total glycoprotein was found to have accumulated (see last column of Table 1).

None of these drugs, nor the physiological stimulant of acid secretion, histamine, nor secretin, had any effect on the acid mucopolysaccharides (bound uronic acids) in these fractions (not shown). However, the average uronic acid found in Fraction I fell from 54 μg to 27 μg per 100 mg wet tissue when the rats were treated with aspirin or atropine (unpublished results).

Table 2. Action of drugs on the incorporation of ^{14}C-glucose into rat gastric mucosal scrapings. Fraction II; mucins released *in vitro*. Fraction III; cell bound glycoprotein. For details, see Methods. The results are given as the average of two separate experiments, each carried out in duplicate. Control, $n = 4$; *i*: inhibition; *a*: activation, both given as per cent of control values

Effector	Dose	Route	Time, min	Radioactivity, d.p.m./mg protein × 10^{-3} Fraction II	*i* %	*a* %	Fraction III	*i* %	*a* %
Control				9.25	—	—	6.25	—	—
Secretin	2 i.u./kg	i.p.	60	9.8	0	—	5.7	15	—
Histamine	100 μg/kg	i.m.	60	6.1	36	—	6.9	—	10
Aspirin	200 mg/kg	per os	15	11.2	—	22	3.0	62	—
Aspirin	200 mg/kg	i.p.	60	10.25	—	11	13.2	—	112
Atropine	2 mg/kg	i.p.	5	11.9	—	29	35.0	—	460

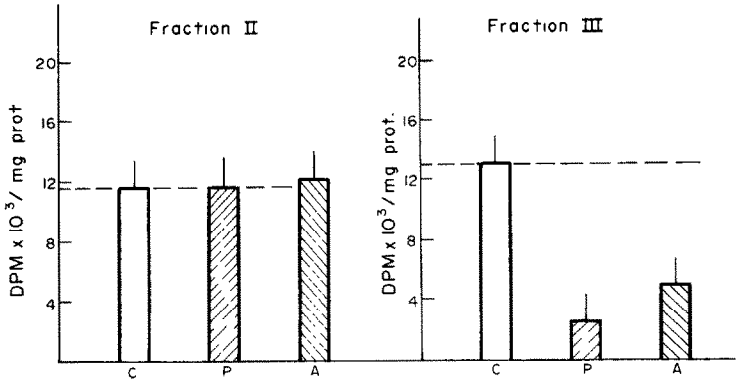

Fig. 2. Incorporation of ^{14}C-glucose in mucins of Fraction II (secreted *in vitro*) and Fraction III (intracellular fraction) of rat gastric mucosa scrapings in the presence of puromycin (P) and actinomycine (A) as compared to controls (C). Radioactivity of fraction as d.p.m./mg protein.

Action on the incorporation of ^{14}C-U-D-*glucose.* The radioactivity of Fractions II and III of the mucosal scrapings from treated rats is compared with that of controls, in Table 2. Secretin had no noticeable effect on the incorporation of glucose in either of these fractions. Histamine administration resulted in a moderate inhibition of glucose uptake in Fraction II. Oral aspirin (15 min) did not affect significantly incorporation in Fraction II but decreased it strongly (−62 per cent) in Fraction III. Parenterally administered aspirin (60 min) did not affect either incorporation in Fraction II but produced a strong increase of incorporation in Fraction III. Atropine (5 min) had an effect similar to, but greater than parenteral aspirin. This activation of glucose incorporation in the cell-bound mucin (Fraction III) contrasts with the strong decrease in the carbohydrate to protein ratio in Fraction I containing *in vivo* secreted, preformed mucin (see Fig. 1).

Puromycin and actinomycin drastically reduced incorporation in Fraction III, but not in Fraction II, as shown in Fig. 2. None of these drugs (aspirin, atropine, puromycin or actinomycin) appeared to affect significantly the incorporation of glucose in Fraction II (secreted mucin *in vitro*).

Effect on sulphate incorporation. Both secretin and histamine injections, as well as the secretion depressants had strong effects on sulphate incorporation into both Fractions II and III (Table 3). It was assumed that all sulphate was bound to the polysaccharide moiety. The scatter was fairly large in most cases, but the decrease in sulphate uptake, particularly in Fraction II ranged from 20–6 per cent of the control values (80–94 per cent inhibition). It is intriguing that histamine and secretin which had no action on glucose incorporation did inhibit sulphate incorporation.

DISCUSSION

The above results can be analysed in terms of the *in vivo* and *in vitro* action of drugs on mucin biosynthesis. Between the moment of their administration and the sacrifice of the animals, the drugs do act *in vivo* on mucin synthesis and secretion as shown by the data concerning Fraction I in Fig. 1. Their action *in vitro* is exemplified by their modification of the incorporation of labelled precursors in Fractions II and III as shown by the data of Fig. 2 and Tables II and III.

The strong reduction of the carbohydrate content of preformed mucin (Fraction I) after oral and parenteral aspirin administration as well as after parenteral atropin administration confirms and extends previous findings by Kent and Allen [1, 4, 5] Kent [16], Rainsford [17] and others concerning the inhibition of mucin formation and/or secretion by these drugs.

In contrast to the above findings *in vivo*, the data shown in Table 1 do not reflect a significant decrease of the carbohydrate to protein ratio in Fraction II mucins (secreted during incubation of the scrapings in the medium) or of the total amount of carbohydrate recovered from Fractions II plus III (extra-

Table 3. Incorporation of $^{35}SO_4^{2+}$ into Fraction II, glycoprotein released into the medium during incubation, and into Fraction III, cell-bound glycoproteins. The animals had been treated *in vivo* as described in Table 2. $n = 53$; *i* per cent = inhibition in per cent of control value

Effector	Route	Time, min	Radioactivity, d.p.m./mg hexose ± S.E. Fraction II	*i*%	Fraction III	*i*%
Control			1320 ± 400	—	420 ± 115	—
Secretin	i.p.	60	240 ± 15	82	170 ± 15	60
Histamine	i.m.	60	200 ± 140	85	70 ± 45	83
Aspirin	*per os*	15	260 ± 35	80	40 ± 30	90
Aspirin	i.p.	60	100 ± 70	92	210 ± 10	50
Atropine	i.p.	5	80 ± 20	92	120 ± 20	71

cellular plus intracellular carbohydrate) in the mucosa suspension from rats treated with atropin or aspirin. In some cases there appears to be even an increase in the carbohydrate content of the extracts obtained from drug treated animals.

The incorporation data of Table 2 confirm these results by showing that glucose incorporation is not inhibited in Fraction II mucins and strongly inhibited only by administered aspirin *per os* in Fraction III (intracellular) mucin. Parenterally administered aspirin and atropin increased substantially glucose incorporation in Fraction III mucin and did not alter it significantly in Fraction II mucin.

The reason for this apparent contradiction between the *in vivo* and *in vitro* results is not yet clear. It may be partially due to a biphasic action observed recently after peroral aspirin administration to rats (Waldron-Edward and Greenberg, unpublished results). After a 10 min inhibition period a strong activation of ^{14}C-adenine and ^{14}C-leucine incorporation was noticed during at least 30 min, accompanied by an increase of O_2 consumption. It is also conceivable that this accelerated synthetic period exhausts the biosynthetic activity of the mucin synthesising cells resulting in a decrease of mucin formation. A decrease of the lysosomal proteolytic and glycolytic enzymes by these same drugs can also contribute to the observed effect (Boutros, Waldron-Edward [18]).

The action of secretin and histamine on mucin synthesis and secretion is less emphatic. The carbohydrate content of the preformed mucin *in vivo* (Fraction I) was not or only slightly affected. The carbohydrate content of excreted mucin *in vitro* (Fraction II) and intracellular mucin (Fraction III) was not affected or even slightly increased by histamin. Secretin did however increase significantly the total amount of mucin recovered from the stomach (unpublished data). This effect is probably strongly time dependent and must be investigated at shorter time intervals.

The action *in vitro* of puromycin and actinomycin strongly suggest the existence of two separate phases of mucin biosynthesis. Both of these drugs severely reduced incorporation of glucose in intracellular mucin (Fraction III). They did not affect glucose incorporation in mucin secreted during incubation (Fraction II) (Fig. 2). No explanation is as yet available for this observation.

Whur, Hercovics and Leblond [19], have suggested that the primary polypeptide with the inner core sugars of the glycoprotein are assembled in the rough endoplasmic reticulum, while the later steps in glycosylation take place in the Golgi apparatus. If one can assume that such a stepwise synthesis takes place in mucin biosynthesis, then it follows that the glycosylation in the Golgi apparatus is not suppressed by puromycin and only partially inhibited by actinomycin, so that ^{14}C-glucose uptake from glucose continues, whereas the biosynthesis of the polypeptide sugar inner core is suppressed by these drugs.

Hog and rat gastric mucins are supposed to be sulphated glycoproteins [5, 20, 21]. This was not definitely shown for human gastric mucin and Lambert *et al.* presented evidence suggesting that the sulphate content of human gastric mucin preparations is due to the admixture of oesophagal or salivary glycoproteins [22]. Sulphate incorporation in rat gastric mucin was shown to be strongly inhibited by all drugs used, in agreement with previous studies on aspirin [17] (see Table 3). These results suggest a much greater susceptibility of the sulphatation stages of mucin biosynthesis to drug action, than those of the glycosylation stages. They also show that the inhibition of sulphatation did not prevent glycosylation or mucin synthesis and excretion by the mucosal preparations. As the physicochemical properties of glycoproteins may be strongly influenced by total negative charge, the physiological properties of undersulphated or unsulphated mucin may differ from those of normally sulphated mucins.

The mucin coating of the stomach provides physical protection for the underlying cells; this layer is lost on ulceration [23]. In the normal unchanged stomach, freshly secreted mucin swells relatively slowly in the course of absorbing luminal fluids; the softened gel then disintegrates and is shed when secretion of acid is stimulated by normal physiological events such as food ingestion; the acidity causes precipitation of the clear mucin gel. The effect of ulcerogens such as aspirin, therefore, may be due either to acceleration of mucin swelling and its subsequent disintegration or else directly on the secretion of the glycoprotein from the surface cells by an initial acceleration of mucin biosynthesis followed by the exhaustion of the mucin synthesising cells.

The described method of analysis illustrates clearly the overall effect of drugs and secretagogues, firstly on the secretion of protective mucin *in vivo*, and secondly, on the cellular response of biosynthesis. However, each of these experiments was restricted to one dose and one time-period of application. The sequence of events which lead to overstimulation and then to tissue breakdown, has yet to be followed and requires further investigation.

REFERENCES

1. D. Snary and A. Allen, *Biochem. J.* **127**, 577 (1972).
2. D. Waldron-Edward and L. Robert, Methodology Structure et Métabolisme des Glycoconjugués, Colloque CNRS No 221, Paris p. 1011 (1974).
3. C. Decaens, D. Waldron-Edward, J. P. Bader and L. Robert, *Conn. Tissue Res.* **4**, 25 (1975).
4. A. Allen and P. W. Kent, *Biochem. J.* **106**, 301 (1968).
5. P. W. Kent and A. Allen, *Biochem. J.* **106**, 645 (1968).
6. C. Decaens, L. Robert, F. Mignon and J. P. Bader, *Biomedicine* **23**, 361 (1975).
7. D. Waldron-Edward, C. Decaens, J. P. Bader and L. Robert, *C.R. Acad. Sci. (Paris)* **279**, 1397 (1974).
8. Z. Dische and L. B. Shettles, *J. biol. Chem.* **175**, 595 (1948).
9. J. Montreuil and G. Spik, Microdosage des Glucides, Monogr. No 1, Fac. Sci. Lille (1963).
10. Z. Dische, *Biochem. Z.* **189**, 77 (1927).
11. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
12. Z. Dische, in *Glick D. Methods in Biochemical Analysis*, Vol. 2. p. 313, Interscience edition (1955).
13. J. Montreuil and N. Scheppler, *Bull. Soc. Chim. biol.* **41**, 13 (1959).

14. D. Waldron-Edward, C. Decaens, J. P. Bader, A. M. Robert and L. Robert, *Pathol. Biol.* **24**, 531 (1976).
15. D. Waldron-Edward, C. Decaens and J. Labat Robert, *Pathol. Biol.* **00**, 000 (1977).
16. P. W. Kent, *Exposés Ann. Biochim. Méd.* **30**, 97 (Masson, Paris) (1970).
17. K. D. Rainsford, in *Agents and Actions*, Vol. 5/4, p. 326, Birkhäuser, Bale (1975).
18. M. R. Boutros and D. Waldron Edward, *Labor. Invest.* **36**, 436 (1977).
19. P. Whur, A. Hercovics and C. P. Leblond, *J. Cell. Biol.* **43**, 289 (1969).
20. D. Waldron Edward, *Can. J. Surgery* **13**, 341 (1970).
21. B. L. Slomiany and K. Meyer, *J. biol. Chem.* **247**, 5062 (1972).
22. R. Lambert, C. Andre and A. Berard, *Digestion* **4**, 234 (1971).
23. R. Lambert, *La Recherche* **45**, 436 (1974).