Asymmetric Distribution of Materials sloughed by Hamster Intestinal Sacs

EVERTED sacs of the small intestine have been used widely for the investigation of transport and metabolism by the small intestine[1]. When such sacs are incubated with a buffer, they slough materials which can interfere with the assay of compounds being investigated and suitable corrections become necessary[2]. Despite this, there have not been any reports as to the nature and distribution of the liberated substances. The present communication gives preliminary data concerning the composition of some of these materials and emphasizes that their distribution is quite asymmetric.

Everted intestinal sacs were prepared from adult golden hamsters (*Mesocricetus auratus*) of both sexes. The animals were allowed food and water freely until they were killed. The small intestine was removed, washed with Krebs–bicarbonate buffer *p*H 7·4, and then everted. Three sacs were made from each small intestine. Individual sacs were filled with 1 ml. of buffer (serosal fluid) and placed in a 25 ml. flask containing 5 ml. of identical buffer (mucosal fluid). After gassing with 95 per cent oxygen (O_2) + 5 per cent carbon dioxide (CO_2), the flasks were stoppered and incubated at 37° C for 1 h. The fluids were drained and centrifuged at full speed in a centrifuge for 20 min in order to remove sloughed tissue. The mucosal and serosal solutions were then handled identically, but separately.

When run at a 1 : 31 dilution (with *p*H 7·4 Krebs–bicarbonate buffer), the average optical densities of the fluids (six sacs) were as shown in Table 1.

Table 1

	Serosal solution	Mucosal solution
260 mμ	0·18	0·07
280 mμ	0·12	0·07

It might be expected that the mucosal side would have a higher concentration of sloughed substances, since the mucosal cells tend to disintegrate histologically during incubation. Yet the optical density was higher in the serosal solution. In terms of total amount, since there was five times as much serosal as mucosal fluid, the mucosal solution contained about two-thirds of the quality of 280 mμ absorbing materials present in both fluids.

One-tenth millilitre of each liquid was dried at 105° C and treated with 3·0 ml. of 0·1 per cent ninhydrin in glacial acetic acid[2]. After 30 min, the optical density was read at 440 mμ. The average of the serosal solutions was 0·62, while the average of the mucosal liquids was 0·19. There was thus a concentration of ninhydrin-reacting materials within the sacs more than three times greater than that in the mucosal fluid.

Following ascending chromatography on Whatman No. 1 paper, strips were air dried and sprayed with 0·1 per cent ninhydrin in butanol. The strips were visually inspected 1 h later, following 2 min of heating at 105° C. Corresponding spots were observed in both mucosal and serosal solutions; there was a much greater intensity of colour with the serosal fluids.

Lipids in mucosal and serosal solutions were also extracted (one part to nine parts of 2 : 1 chloroform–methanol) and spotted on glass plates that had been coated with 250μ of activated 'Absorbosil-1'. Thin layer chromatography was performed in a solvent system of petroleum ether (boiling point 30°–60°): diethyl ether (peroxide free) : glacial acetic acid, 90 : 10 : 1. After 45 min the plates were air-dried and stained with iodine vapour. Spots were outlined with a pencil and a tracing made. Diglycerides, cholesterol, unsaturated fatty acids, saturated fatty acids, triglycerides and cholesterol esters were identified by their R_F values (and comparison with standards). As judged by the intensity of the iodine stain, serosal fluids contained less triglyceride than the mucosal solution. The serosal fluid, however, appeared to contain more fatty acid. This suggests that triglycerides were being split by the intestinal sacs, with fatty acids then entering the serosal fluid.

Table 2

Solvent system	R_F values of principal ninhydrin-reacting spots
Butanol : acetic acid : water (4 : 1 : 1)	0·08 maroon 0·20 red purple 0·26 purple 0·34 yellow 0·46 purple 0·58 purple
Phenol saturated with water	0·84 yellow with violet border
Butanol : ammonium hydroxide (4 : 1)	0·08 yellow 0·12 violet 0·16 violet 0·37 violet 0·48 violet

Aliquots of the lipid extracts were evaporated at 4° C and an aliquot was transmethylated for 6 h at reflux temperature using 5 per cent sulphuric acid in anhydrous methanol. The fatty acid composition was analysed by using the resultant methyl esters and gas-liquid chromatography (see Table 3). Peaks were compared with known standards. The composition of the large "unknown" peak from the serosal fluid is uncertain. Its retention time on the chromatographic column was 4·8 times that of stearic acid and may represent oxidized fatty acids.

Table 3

Fatty acid	Mucosa (per cent)	Serosa (per cent)
14 : 0	3·2	2·2
16 : 0	20·6	17·2
16 : 1	3·9	4·0
18 : 0	10·9	7·2
18 : 1	24·2	14·8
18 : 2	23·1	12·1
18 : 3	3·2	3·6
20 : 0	—	1·7
20 : 4	1·8	1·4
Unknown	9·0	35·7

Asymmetric distribution of materials sloughed by intestinal sacs *in vitro* may be a manifestation of continuing transport from the mucosal surface toward the serosal surface. In addition, any persistent electrical potential difference across the sacs (mucosa negative with respect to the serosa) would also contribute to an asymmetry of the materials.

This work was supported by grants from the U.S. Public Health Service.

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Influence of the Oviduct and Ovum on Cyclic Ovarian Activity in the Rat

THE muscular and secretory activity of the female genital tract has been implicated in the processes of gamete transport with emphasis on the role of the uterus and oviduct. It is well known that the physiological activity of these two portions of the female genital tract varies with respect to the hormonal phases of the ovarian cycle[1]. The ampullary portion of the oviduct plays still another important part as the site of fertilization in most mammals. In spite of the wealth of information concerning mechanisms of tubal transport, there has been a lack of knowledge concerning any additional interactions between the oviduct and ovary. It has been reported by Bishop[2] that secretions of the oviduct undergo changes in response to cyclic ovarian activity.

One major characteristic of mammalian ovarian cycles is the shedding of the ovum at the time of ovulation, and these ova, whether fertilized or not, are transported into