periment. On the other hand, electron microscopic findings obtained in the present study demonstrated that, at the acute stage after the BC treatment, smooth muscle cells suffered from injurious effect of the agent as did nerve cells, but that smooth muscle cells recovered fully and nerve tissues alone were irreversibly damaged at the chronic stage. Thus, it may be reasonable to suppose that the observed selective destruction of nervous elements of the intestinal wall, undergoing the BC treatment, was partly the result of poorer repairing ability of nervous tissues as compared with other tissues such as smooth muscles.

The fact that BC solution, under certain conditions, may exert considerable injurious effects on the intestinal wall is to be emphasized as a warning, since the drug is commonly used as an antiseptic in the operating room. On the other hand, the agent may be profitably utilized in research fields as a simple means of denervating certain tissues without injuring other tissue constituents.

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Radioimmunoassay of progesterone in uterine flushings of buffalo *(Bubalus bubalis)*

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Summary. The concentration of progesterone as determined by radioimmunoassay varied in accordance with the phase of ovarian activity.

The significant role played by uterine fluid in sperm capacitation, nourishment, development and implantation of embryo is well recognized. As far as qualitative and quantitative aspects are concerned, very little information was available on its ovarian steroid contents. The progesterone content of rabbit uterine fluid has very recently been shown to play some role in implantation of blastocyst[3-5]. However, no report could be found on qualitative and quantitative aspects of this hormone in the buffalo. The present investigation was a step towards screening this hormone in uterine fluid of buffaloes during various phases of ovarian activity.

Materials and methods. Sample collection: 23 genitalia from apparently healthy buffalo cows were collected from the abbatoir. All necessary precautions were made to prevent contamination of uterine fluid with any other fluid, viz. oviducal, cervical and the blood. The organs were classified into follicular, subactive and luteal phase by gross morphological examination of ovary[6]. Each horn was flushed with 20 ml normal saline and the flushings stored at $-20\,^{\circ}C$ pending analysis.

Analysis. Samples were thawed at room temperature. The fluid was centrifuged under refrigeration at 12,000 rpm for 10 min, to remove the cellular debris. The progesterone was assayed in duplicate in 0.5 ml of the supernatant by the method of Aso et al.[7]. The sensitivity of the assay in term of detection limit was 4.70 pg. The coefficient of intra assay variation carried out in the same assay using pooled uterine fluid was 17.3%. The specificity of antiserum has already been reported from this laboratory (Arora et al.[8]).

Results and discussion. The progesterone concentration during follicular, subactive and luteal phases, was observed to be 118.12 ± 6.6, 187.76 ± 23.55 and 782.50 ± 117.89 pg/ml, respectively (table).

The calculated level of progesterone is likely to be a substantial underestimate, since the recovery of the fluid was not 100%. Our values during the luteal phase are quite low compared with rabbits[3] during early pregnancy. The difference is likely to be the species difference. The source of progesterone in the uterine fluid has been shown to be the blood plasma[9]. Patek and Watson[10], however, demonstrated the synthesis of progesterone by sow endometerium. The concentration of progesterone during luteal phase was 5-8 times higher as compared to follicular phase. Batra et al.[11] reported about 5-10 times higher values of progesterone in these 2 phases during oestrous cycle in buffalo. It may therefore be concluded that the source of progesterone in uterine fluid of buffalo is also the blood plasma. At the same time, the active secretion from the endometerium cannot be ruled out.

The progesterone content has been demonstrated in pig blastocysts[12,13]. Rabbit blastocysts have also been shown to undergo steroid conversions[14]. Seamark and Lutwak-Mann[15], however, believed that the progesterone may not necessarily be synthesized by blastocysts but may be conveyed to them by endometerial secretions. The progesterone from the rabbit maternal tissue to the embryo was thought possibly to be carried with a protein, uteroglobin, where it stimulates cell divisions and blastocyt expan-

Progesterone concentration (pg/horn) in uterine fluid during various phases of ovarian activity

Follicular phase	Luteal phase	Subactive phase
96.50	435.0	71.00
108.00	506.50	74.00
120.50	575.00	105.50
130.50	742.00	119.00
111.50	1041.00	122.00
141.75	1060.00	246.00
	1107.50	269.00
		284.00
		400.00
118.12 ± 6.6	782.50 ± 117.89	187.76 ± 23.55

$\pm$ SEM.

sion[16,17]. In view of the above observations, and the higher concentration of progesterone recorded during luteal phase there is the possibility of its role in the implantation of the blastocyst during early pregnancy in buffaloes also.

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In vitro esterification of plant sterols by the esterifying enzyme of the small intestine of rat[1]

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Summary. The in vitro esterification of plant sterols, β-sitosterol, campesterol and stigmasterol, by the esterifying enzyme of the small intestine of rat was studied in the presence of saturated and unsaturated fatty acids. Campesterol esterification was highest, followed by sitosterol and stigmasterol irrespective of the type of fatty acid. Both campesterol and sitosterol esterification was greater with unsaturated fatty acids than with saturated fatty acids.

The intestinal absorption of plant sterols is considered practically negligible[2-5]. Thus, only minute amounts of plant sterols are detectable in blood and tissues[2-6], although the usual diet may contain appreciable amounts of these sterols[7]. 5 years ago, a new inherited lipid storage disease involving plant sterols was reported in humans[8]. In the disease called 'β-sitosterolemia and xanthomatosis', considerable amounts of plant sterols, β-sitosterol in particular, were found in the blood and tissues of the patients[8,9].

One cause of the disease was found to be greatly increased intestinal absorption of sitosterol[8,10]. After reviewing the events involved in sterol absorption in the small intestine, Bhattacharyya and Connor[8] suggested that enhanced absorption of plant sterols in the new disease probably involved the esterification and incorporation of the plant sterols into chylomicrons. This implied that the enzyme responsible for esterification of cholesterol (cholesterol esterase) within the intestinal mucosa may have lost its specificity for structural requirement of the sterol substrate and acts upon plant sterols thereby facilitating abnormally the absorption of the latter. Since no information is available regarding the esterification of plant sterols by the intestinal esterifying enzyme, the present study was undertaken to study the esterification of plant sterol, β-sitosterol, campesterol and stigmasterol, using acetone-dried powder preparation of the enzyme from the rat intestine.

Materials and methods. Plant sterols, cholesterol and fatty acids (Applied Science Labs., State College, Pa.) used in the study were between 97 and 99+% pure and used without further purification.

Preparation of acetone-dried powder of the intestinal esterifying enzyme. 5 normal Sprague-Dawley rats maintained on regular diet (Purina rat chow, Purina Ralston Co., St. Louis, Mo.) were killed and the small intestine was removed quickly, stripped off of any mesentary and collected in ice-cold saline. The intestine was washed thoroughly with ice-cold saline, blotted on filter paper and placed in 10 volumes of acetone pre-cooled to −15 °C. The tissue was homogenized in a Virtis homogenizer and acetone-dried powder was prepared[11]. The powder was suspended in cold distilled water in the proportion of 1 g powder per 10 ml water for 1 h and centrifuged at 4 °C at 10,000×g for 10 min. The clear supernatant was suitably diluted and was used as the enzyme source.

Assay of the esterifying enzyme activity. The incubation mixture for the assay of the esterifying enzyme activity contained in a total volume of 5 ml, 5 μmoles of the free sterol and 10 μmoles of the given fatty acid in 0.5 ml ethanol, 3 ml of potassium phosphate buffer, 0.1 M, pH 6.1, 25 mg of sodium taurocholate and the enzyme extract representing about 25 mg of acetone-dried intestinal powder containing about 8 mg protein. The incubation was carried out in a metabolic shaker incubator at 37 °C for 1 h. Reactions were terminated by adding 5 ml ethanol to the system and total and free cholesterol determined before

Plant sterol and cholesterol esterification by the esterifying enzyme of the small intestine of rat

	Fatty acids			
	Palmitic 16:0*	Stearic 18:0	Oleic 18:1	Linoleic 18:2
Plant sterols				
β-Sitosterol	1.04±0.6	1.10±0.6	4.71±1.1	4.71±1.3
Campesterol	3.74±1.0	3.49±1.1	8.53±1.7	8.16±1.9
Stigmasterol	0.47±0.3	0.55±0.2	0.94±0.3	0.59±0.3
Cholesterol	6.7 ±0.8	6.5 ±0.9	41.7 ±2.6	88.6 ±6.7

Values are mean±SD of 4 determinations and expressed as nmoles esterified/mg protein/h. The assay system contained in a total volume of 5 ml: approximately 25 mg intestinal powder containing about 8 mg protein; potassium phosphate buffer 0.1 M, pH 6.1, 3 ml; sodium taurocholate 25 mg; sterol, 5 μmoles and fatty acid, 10 μmoles. * No. of carbon atoms: no. of double bond.