To surmount some of the shortcomings of the ligated loop technique, Thiry-Vella loops were prepared in calves, and the effect of enterotoxin on transfer of fluid, glucose and electrolytes was observed.

Enterotoxin was prepared as described by Smith & Halls. Volumes (30 ml) of culture filtrate were precipitated with 8 volumes of acetone, and the resulting precipitate was redissolved in 30 ml of an electrolyte solution containing polyethylene glycol 4000 as a marker. This was the standard amount used in the present experiments. A control solution contained equivalent amounts of extract from uninoculated culture medium. The solutions containing extract were approximately isotonic.

The net absorption of fluid, sodium, potassium, bicarbonate, chloride and glucose was observed during a control period, and again during an immediately subsequent period in the presence of enterotoxin. Control experiments showed that absorption during such consecutive periods was the same in the absence of enterotoxin.

In each of eight loops examined, the presence of enterotoxin caused net secretion of fluid and sodium ($P<0.05$). Loops which absorbed during the control period began to secrete, while those which secreted during the control period showed an increased secretion. Similarly in four loops examined for net chloride and bicarbonate transport, the presence of enterotoxin caused a shift towards secretion in each case ($P<0.05$). Potassium absorption was significantly affected in only two of eight loops, while glucose absorption was unaffected in all of eight loops examined.

The effect on net sodium and fluid absorption could have resulted from increased secretion, decreased absorption or both. In order to define the effect more closely, ^{22}Na and deuterium oxide were used as isotopic labels to determine the unidirectional fluxes of sodium and fluid during 10 min periods following exposure to control and enterotoxin solutions. The flux from the lumen was termed insorption, and the flux towards the lumen exorption (Code, 1960).

In the first of two loops in which the unidirectional fluxes were examined, it was found that the presence of enterotoxin caused increased exorption of sodium ($P<0.05$). The insorption of sodium was not significantly changed. In the second loop, however, the sodium insorption was decreased ($P<0.05$) while the exorption was increased ($P<0.05$).

In the case of fluid transfer, in the first loop neither the small decrease in insorption nor the small increase in exorption was itself significant, despite a net shift towards secretion ($P<0.05$). In the second loop, however, there was an increased exorption of fluid ($P<0.05$) although the insorption was again not significantly altered.

Experiments on four loops in the presence of enterotoxin confirmed that either sodium insorption or exorption could be altered, but the effect on fluid movement in all four was to increase significantly exorption while leaving insorption unchanged.

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The origin of ascorbic acid stored in the leucocytes

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Denson & Bowers (1961) demonstrated that ascorbic acid is actively concentrated

into leucocytes and erythrocytes contain low concentrations of ascorbic acid (Crandon, Lund & Dill, 1940; Butler & Cushman, 1940; Butler, Cushman & MacLachlan, 1943). Dehydroascorbic acid enters the erythrocytes more rapidly than ascorbic acid (Hughes & Maton, 1968). Little is known about the concentrating mechanisms in the leucocytes or about the relationship between ascorbic acid levels in the erythrocytes and leucocytes. Standard counts of human leucocytes have been incubated at 37° C in 3 mg ascorbic acid per 100 ml isotonic phosphate buffer at pH 7·4, and ascorbic acid concentrations have been measured by the dinitrophenyl hydrazine method.

Ascorbic acid concentrations in leucocytes increased significantly after 4 h incubation at pH 7·4 (Table 1). Alteration of the pH to 7·8 reduced the absorption and

TABLE 1. *Leucocyte ascorbic acid concentrations* ($\mu g/10^8$ *cells*) *after incubation at* 37° *C in ascorbic acid* 3 *mg*/100 *ml under various conditions*

pH	No. of observations	Control (mean ± S.E.M)	2 h (mean ± S.E.M)	4 h (mean ± S.E.M)
7·4	7	34·74 ± 4·12	67·83 ± 7·91	81·72 ± 5·72
7·8	7	34·24 ± 4·12	46·88 ± 5·35	41·50 ± 3·76
5·4	7	34·24 ± 4·12	63·04 ± 7·09	104·46 ± 12·46
7·4 Control	5	53·32 ± 4·21	51·24 ± 4·64	55·18 ± 4·49
7·4 + ATP	5	35·32 ± 4·21	57·09 ± 4·92	67·91 ± 5·30
7·4 + O_2	5	35·32 ± 4·21	62·86 ± 4·01	69·68 ± 5·36
7·4 + O_2 and ATP	5	35·32 ± 4·21	79·06 ± 3·35	79·56 ± 7·56

rate of uptake of ascorbic acid; at pH 5·4 the rate of uptake was the same as at pH 7·4 at 2 h, but the total absorption in 4 h was significantly higher than at pH 7·4. Absorption was significantly increased after the cells had been incubated with ascorbic acid in the presence of adenosine triphosphate (ATP), or in oxygen. Oxygen and ATP together further increased the absorption of ascorbic acid, and its maximum uptake had been attained after 2 h incubation. It can therefore be concluded that uptake by the leucocytes is an active energy requiring process. 2 : 4 Dinitrophenol reduced but did not prevent the uptake, indicating that the leucocyte ascorbic acid uptake may involve both active and passive adsorption (Milne, 1964). Incubation of leucocytes alone, in the presence of dehydroascorbic acid, did not affect their ascorbic acid concentration. However, incubation of erythrocytes in dehydroascorbic acid significantly increased the concentration of ascorbic acid in the supernatant fluid (0·29 ± 0·020, rising to 0·50 ± 0·037 mg/100 ml in 2 h) and incubation of erythrocytes together with leucocytes resulted in a reduction of this increased level of ascorbic acid in the supernatant fluid (0·35 ± 0·020 ml).

It can therefore be concluded that uptake of ascorbic acid by the leucocytes involves an active and a passive process which takes place maximally at acid pH. Leucocytes have an ascorbic acid storage function, the ascorbic acid originating from dehydroascorbic acid manufactured by the erythrocytes.

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