anti-inflammatory drugs and also from rats with adjuvant arthritis showed reduced turbidity on heating. The present studies define the stages in the development of the arthritis when the immunosuppressant azathioprine is effective in suppressing symptoms and explore the relationship between the appearance of swelling and of serum changes in arthritic controls and in similar rats also given either azathioprine or phenylbutazone.

Arthritis was induced in male Wistar rats by injection of 0·1 ml Freund's adjuvant into one hind-foot pad. A primary swelling in the injected foot was seen within about 24 h while secondary, arthritis-like lesions appeared in the other feet about 14 days later.

Azathioprine completely prevented secondary swelling but had no effect on primary lesions when oral doses of 25 mg/kg were given daily from the day of adjuvant injection (day 0) until day 10. There was marked reduction in secondary swelling when the drug was given only on days 0–4, 2–6 or 4–8 and slight reduction with treatment on days 8–12. Dosing after day 12 was ineffective.

To examine the serum changes blood was drawn from the tail on alternate days after adjuvant injection; 0·1 ml of serum from each rat was diluted with 2·9 ml M/15 phosphate buffer and heated at 69° C for 30 min. The resulting turbidity was measured on a spectrophotometer. In arthritic controls where was an approximately 50% decrease in the turbidity measurement by day 2. The value remained low during the course of the arthritis and often dropped further around day 14. The drop was related to the severity of the ensuing arthritic lesions and rats which did not develop arthritis showed normal serum turbidity. Adjuvant-injected rats given azathioprine (25 mg/kg) daily from days 0–10, although protected against arthritis, also showed lowered turbidity measurements, but at 21 days the drop was less than in arthritic controls ($0{\cdot}01<P<0{\cdot}05$). When phenylbutazone (100 mg/kg) was similarly administered the appearance of secondary arthritic swelling was largely prevented while primary swelling was reduced. Turbidity changes resembled those in arthritic controls on days 2 and 4, but were significantly less than in arthritic controls from day 7 onwards ($P<0{\cdot}1$) and from day 11 the values were not significantly different from those in untreated controls.

REFERENCES

GRAEME, M. L., FABRY, E. & SIGG, E. B. (1966). Mycobacterial adjuvant periarthritis in rodents and its modification by anti-inflammatory agents. *J. Pharmac. exp. Ther.*, **153**, 373–380.

NEWBOULD, B. B. (1963). Chemotherapy of arthritis induced in rats by mycobacterial adjuvant. *Br. J. Pharmac. Chemother.*, **21**, 127–136.

PEARSON, C. M. (1956). Development of arthritis, periarthritis and periostitis in rats given adjuvants. *Proc. Soc. exp. Biol. Med.*, **91**, 95–101.

PILIERO, S. J. & COLOMBO, C. (1967). A new in vivo assay for screening drugs for potential anti-inflammatory activity. *Clin. Pharmac. J. new Drugs*, **7**, 198–206.

WARD, J. R., CLOUD, R. S., KRAWITT, E. L. & JONES, R. S. (1964). Studies on adjuvant induced polyarthritis in rats. III The effect of "immunosuppressive agents" on arthritis and tuberculin hypersensitivity. *Arthritis Rheum.*, **7**, 654–661.

Some effects of *Escherichia coli* enterotoxin on fluid and electrolyte transfer in calf small intestine

R. J. BYWATER (introduced by F. ALEXANDER), *Department of Veterinary Pharmacology, Royal (Dick) School of Veterinary Studies, University of Edinburgh*

Certain enteropathogenic strains of *Escherichia coli* produce an enterotoxin which causes dilatation of ligated loops of intestine (Smith & Halls, 1967).

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.

REFERENCES

CODE, C. F. (1960). The semantics of the process of absorption. *Perspect. biol. Med.*, **3**, 560–562.
SMITH, H. W. & HALLS, S. (1967). Studies on *Escherichia coli* enterotoxin. *J. path. Bact.*, **93**, 531–543.

The origin of ascorbic acid stored in the leucocytes

H. S. LOH* and C. W. M. WILSON, *Department of Pharmacology, Trinity College, University of Dublin*

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