Substitution of gluconate for carbonate as the anion in the calcium salt resulted in no significant difference in metabolic effect, as was anticipated from the results of Patton and Sutton (1952). The change from carbonate to gluconate was attempted because carbonate caused a degree of constipation and abdominal distress which was absent when gluconate was employed. The gluconate, however, because of the higher molecular weight, requires considerably more of the salt to achieve the same calcium level, presenting the problem of larger numbers of tablets to be ingested.

The lowering of serum cholesterol and triglycerides by calcium is interesting from two points of view. First, Wells and Cooper (1958) reported that calcium inhibited cholesterol absorption in rats in the presence of dietary fat. Calcium was also reported to be hypocholesterolaemic in rabbits (Iacono *et al.*, 1960). This might in part explain some of the hypocholesterolaemic activity of calcium in that absorption of exogenous cholesterol may have been inhibited. The second reason for interest in the hypolipaeic action of calcium was the repeated reports of a negative relation between mortality from cardiovascular disease and the hardness of drinking-water (Schroeder, 1960a, 1960b; Morris *et al.*, 1961). Since calcium is the major cation associated with water hardness, it was not unexpected that a similar negative correlation was found between water calcium-ion concentration and cardiovascular disease mortality.

Studies are being conducted into the mechanism of the hypolipaeic effect of calcium and upon the effects of calcium ingestion upon tissue lipids. It is anticipated that this preliminary study will be expanded to examine the effects of dietary calcium on larger numbers of people, particularly young males with electrocardiographically proved myocardial infarctions, in order to ascertain whether a significant decrease in morbidity and mortality could be attained in this way.

Summary

Increased calcium ingestion by subjects of both sexes who were free of clinical coronary heart disease caused a significant decrease in both serum cholesterol and serum triglyceride levels. Both faecal dry matter and total faecal lipids were increased. The exact mechanism of the hypolipaeic action of calcium still requires clarification.

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Indomethacin: Studies of Absorption and of the Use of Indomethacin Suppositories

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Indomethacin, a new drug for treating arthritic disorders, was introduced for clinical trial in 1962. It is chemically unrelated to either corticosteroids or other anti-inflammatory compounds, and has an indole structure within its formula [1-(p-chlorobenzoyl)-5-methoxy-2-methylindol-3-acetic acid].

Although anti-inflammatory activity can be demonstrated in animals (Winter, Risley, and Nuss, 1963), this effect has not been conclusively shown in man, though measurable reduction in the finger-swelling of patients with rheumatoid arthritis, as measured by jeweller's rings, occurs (Hart and Boardman, 1963). Other parameters of inflammation such as the erythrocyte sedimentation rate are unaffected.

The reports of controlled clinical trials (Hart and Boardman, 1963; Wanka, Jones, Wood, and Dixon, 1964; Percy, Stephenson, and Thompson, 1964) prove that this drug relieves symptoms in different arthritic disorders such as rheumatoid arthritis, osteoarthritis, ankylosing spondylitis, and gout. Unfortunately, like other analgesic and anti-inflammatory

agents, it carries the risk of gastro-intestinal irritation. The tablets which were used originally led to peptic ulceration in some patients (Catoggio, Centurion, Alberti, Roldan, and Canepa, 1964; Kelly, 1964; Lövgren and Allander, 1964; Robinson, 1964; Wanka *et al.*, 1964), and anorexia, nausea, vomiting, dyspepsia, and diarrhoea have also occurred. Other side-effects have involved the central nervous system (headache, giddiness, faintness, mental changes, drowsiness, and blurring of vision).

The introduction of gelatin capsules containing powdered indomethacin and the prescribing of smaller doses have led to a decreased incidence of side-effects. However, we thought it important to assess its absorption from the rectum in the hope that suppositories could be used for patients suffering from peptic ulcers, for those who develop gastric intolerance to the drug, and for patients who require larger doses than can safely be given by mouth.

Materials and Methods

Of 40 patients studied, 34 suffered from rheumatoid arthritis, 3 from osteoarthritis, 2 from ankylosing spondylitis, and 1

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from gout. Many were severely ill or resistant to other drugs, or had developed complications of corticosteroid therapy.

The average dose of indomethacin, as capsules, is 25 mg. four times a day. It was decided to compare initially the serum levels and clinical response after giving 100 mg. either as a single oral dose or as a suppository in 10 patients. The capsules or suppositories were given one-half to one-and-a-half hours after a normal breakfast.

Estimation of Indomethacin

The following reagents were used.

1. *Heptane* was washed successively with *N* NaOH, *N* HCl, and distilled water in this order, and passed through filter-paper to remove residual water.

2. *Citrate buffers* (pH 5): Strength (1) molar (citric acid 210 g., NaOH 90 g., H₂O to 1,000 ml.). (2) 0.2 molar.

3. *Phosphate buffer* (pH 8): Disodium hydrogen phosphate (Na₂HPO₄·2H₂O), 16.9 g.; potassium dihydrogen phosphate (KH₂PO₄), 0.68 g.; water to 1,000 ml.

4. *Stock solution indomethacin* (25 µg./ml.). Indomethacin 25 mg. was dissolved in 1,000 ml. of the phosphate buffer. This required continuous stirring for four to five hours and standing for 24 hours at 4° C. before use.

The following method of estimating indomethacin in the serum, modified from that of the manufacturers, was used. One millilitre of serum was pipetted into a glass-stoppered centrifuge-tube containing 2 ml. of *M* citrate buffer (pH 5) and 25 ml. of heptane containing 5% isoamyl alcohol. The contents of the tube were mechanically shaken for 15 minutes and then centrifuged. Twenty millilitres of the upper heptane phase was pipetted into a second centrifuge-tube containing 5 ml. of 0.2 *M* citrate buffer (pH 5). This was shaken for a few minutes and then centrifuged. Ten millilitres of the heptane phase was pipetted into a third centrifuge-tube containing 3 ml. of 0.1 *N* NaOH and shaken for five minutes. After centrifuging, the lower aqueous phase was aspirated into a quartz cuvette and read in an Aminco-Bowman spectrophotofluorometer (activation about 300 mµ, fluorescence about 385 mµ, the exact peaks for each individual machine being found by scanning). Standards were set up by diluting the stock indomethacin solution with distilled water and adding this to normal serum to give concentrations of 0, 2.5, and 5 µg. of indomethacin per ml. These sera can be kept by deep-freezing, but the stock indomethacin solution deteriorates. The graph of concentration against fluorescent reading was linear, and only three points were necessary to form each calibrating graph.

The method is not difficult and is both accurate and precise, provided care is paid to details; for example, traces of alkali remaining from routine laboratory washing procedures may interfere. Indomethacin is light-sensitive, so that prolonged exposure to daylight or delay in taking readings in the spectrophotofluorometer must be avoided. Erroneous results occurred

FIG. 1.—Serum indomethacin levels in unknown paired aliquots

D

if the patient was taking aspirin, for salicylates are also extracted by this technique and fluoresce at a similar wavelength; paracetamol does not interfere. The accuracy is shown in Fig. 1, where aliquots were randomized and then estimated serially.

The distribution of paired samples is of a homo-scedastic nature, and thus the percentage error will be relatively greater when estimating lower concentrations of indomethacin. A single reading has a 95% chance of being within 0.1 µg. of the true value.

The clinical effectiveness in all 40 patients was assessed by relief of pain, improvement of function, and by the cuff dolorimeter, a device for measuring tenderness of the finger-joints (to be published); as well as by the reduction of other therapy such as corticosteroids. No patient was considered to have responded to indomethacin unless relapse took place when dummy capsules or suppositories were substituted without the patient's knowledge.

Results

Indomethacin was absorbed satisfactorily from the rectum. The peak levels obtained varied widely between individuals, being 1.1–3.5 µg./ml. (mean of 10 cases, 2.15 µg./ml.) after 100 mg. of indomethacin by rectum and 1.4–6.2 µg./ml. (mean of 10 cases, 2.85 µg./ml.) after 100 mg. orally. It will be seen (Fig. 2) that the serum level in the average case after 100 mg. by rectum was 75% of that when given by mouth.

FIG. 2.—Average serum levels obtained after giving indomethacin by mouth and by rectum.

Absorption invariably started shortly after a suppository, though it was delayed to a varying extent, up to two hours, after the capsules (Fig. 3); indeed, in five patients it had not begun at 20 minutes. Symptomatic relief showed a similar pattern to the serum levels, starting 20 to 30 minutes after a suppository and 40 to 60 minutes after oral administration (Fig. 4). A curious and unexplained finding was the duration of relief of pain from a suppository in some patients. When 100 mg. was given last thing at night, not only was early-morning stiffness relieved but pain was lessened until midday,

FIG. 3.—Results in a woman aged 49, showing that absorption may be delayed up to two hours after oral administration.

up to 16 hours. Proof when relying on subjective phenomena is always difficult. No such difficulty was found with placebo suppositories; indeed, the relapse was usually obvious next morning.

There was no practical difficulty in insertion of the suppositories except in patients with severe deformity of the hands, when a nurse or relative could do this instead. Patients pre-

FIG. 4.—Effect of indomethacin upon joint tenderness as shown by the cuff dolorimeter. B shows the decrease in tenderness of a proximal interphalangeal joint after 100 mg. by mouth, the corresponding serum level being shown in D. A shows the decrease in tenderness of the same joint after 100 mg. by rectum, the serum level being shown in C.

ferred to use the suppositories at night, as they were easily retained and were unaffected by defaecation, which is usually a morning habit. Local effects from the suppositories were noted in three cases. Sigmoidoscopy was performed in 10 patients, and the rectal mucosa was normal. Occult blood tests on the stools, performed routinely, showed no evidence of bleeding.

No dyspeptic symptoms occurred when suppositories were used, and systemic side-effects such as headaches or dizziness were negligible.

Thirty (75%) of the 40 patients given suppositories obtained a good result, and the clinical effects were similar whether the drug was given by mouth or by rectum. Certain patients who failed to respond to a daily dosage of 100 to 150 mg. were given larger doses, up to 300 mg. daily, by supplementing the oral doses with suppositories. This procedure might be effective, though it was more likely to produce side-effects.

Isolated serum levels were found to be of no practical value in controlling treatment. The peak serum level after rectal administration closely corresponded to one hour, though after oral administration it was too variable to be predictable. The maximum serum levels obtained in different patients did not equate with the degree of relief of pain or the severity of side-effects in these patients.

Discussion

This trial has shown that indomethacin is a valuable drug for the treatment of patients with various types of arthritis. Relapse when a dummy tablet or suppository is substituted is striking and sometimes suggests a "rebound effect." Its use may enable corticosteroid therapy to be reduced in some patients and occasionally to be discontinued altogether.

Capsules are suitable for routine use, although suppositories can be prescribed for the following reasons: (1) when oral administration is contraindicated because of dyspepsia or peptic ulceration, and (2) to increase the total daily dosage above limits now regarded as safe when given by mouth (such as 150 mg. daily).

Side-effects have been no more than an inconvenience, and, in particular, no gastric disturbance has occurred in our trial. It is not yet known why oral indomethacin, like other analgesic or anti-inflammatory drugs, may injure the stomach. If the disturbance is due solely to local damage to the mucosa it should be avoided by rectal administration. If, however, indomethacin causes an increase in the hydrochloric-acid secretion or a decrease in mucosal resistance from a systemic action, there may still be a risk of this after suppositories.

Summary

The estimation of serum levels of indomethacin has shown that it is absorbed satisfactorily from the rectum.

Suppositories containing this drug can be used when capsules are contraindicated because of peptic ulcer or to supplement the total daily dosage.

Indomethacin is a valuable drug for the treatment of patients with various types of arthritis.

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Adrenocortical Carcinoma Treated with *o,p'*-DDD

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The modern chemotherapy of adrenocortical carcinoma stems from the observation by Nelson and Woodard (1949) that the insecticide DDD¹ induced a selective necrosis of the zona

¹ TDE is now the British standard name for DDD.

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fasciculata and zona reticularis of the adrenal cortex in dogs. Initial efforts to use it in man were unsuccessful (Sheehan *et al.*, 1953; Törnblom, 1956; Zimmermann *et al.*, 1956). However, the subsequent discovery that a contaminant of crude DDD, the ortho prime isomer *o,p'*-DDD (2-(2-chlorophenyl)-2-(4-chlorophenyl)-1,1-dichlorethane) (Fig. 1), was a much more effective drug, led to further investigation of its action in the treatment of adrenocortical cancers. Reports of its use in a