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SYMPOSIUM

The toxicity of analgesic substances

Report of a Symposium organised by the Department of Pharmaceutical Sciences of the Pharmaceutical Society of Great Britain, and held in the Society's House, 17 Bloomsbury Square, London, W.C.1, on February 23, 1966.

CHAIRMAN: J. M. Robson

Chairman's Introduction

In this Symposium we are dealing with a series of drugs which is probably more important than any other in medicine. I refer not only to potent analgesics, but also to the minor analgesics. These are very important because they do much to make life easier for many people who suffer from ill health and various types of pain. I think it is true to say that until recently this group of substances was regarded as reasonably non-toxic, and they have been used on a tremendous scale, with that idea being more or less accepted. What has modified our attitude is the experience obtained with new drugs in the last decade. The organic chemist has produced many new compounds, which have been tested in animals and then in man, and a few have been accepted as therapeutic agents, but even after testing for long periods to the limit of possibilities in animals, and extensively in man, with more experience new toxic effects have been revealed. One can give many instances of this, but of course the thalidomide story is itself the best example.

This has made us more aware of the possibility that drugs which have been used for many years might have serious toxic effects, and the purpose of this symposium is to try and assess to what extent various drugs have potential toxic effects. As well as being concerned with the true assessment of these toxic effects, I think implied in our mandate is the question, if such is the case, should we do anything about it?

J. Pharm. Pharmac., 1966, **18**, 331–353

The nephrotoxicity of analgesics

L. F. PRESCOTT

IN recent years there has been increasing suspicion that prolonged or excessive use of analgesics may result in progressive renal damage very similar to that produced by chronic pyelonephritis. Phenacetin (acetophenetidin) has been widely incriminated as the offending drug, mainly because it was common to all the analgesic mixtures mentioned in the early reports. Histologically and functionally the renal lesions are predominantly tubular, with interstitial fibrosis, tubular degeneration and atrophy and a high incidence of papillary necrosis and pyelonephritis (Rubenstein, Abrahams, Stables & Levin, 1964).

PHENACETIN

Although phenacetin has been present in analgesic mixtures abused in most recent reports of chronic interstitial nephritis, no case has yet been reported in which phenacetin was the only drug taken. Other drugs, including the salicylates, the antipyrine group, caffeine, codeine and barbiturates have also been taken concurrently, and the potential toxicity of these other drugs does not seem to have been adequately assessed. It is also possible that drugs other than those already mentioned may cause renal damage after prolonged use. Kasanen & Vasama (1964) noted prolonged use of primidone (Mysoline) and corticosteroids respectively in two patients with unexplained papillary necrosis, and only 14 of the 44 cases of chronic interstitial nephritis reported by Spühler & Zollinger (1953) gave a history of abuse of analgesics. The latter authors felt that sulphonamides and antibiotics were responsible for the renal lesions and in fact did not even mention phenacetin. Similar lesions appear to arise spontaneously in cats (Lucke & Hunt, 1965), mice and rats (see Studer, 1965).

While the "common denominator" theory may implicate phenacetin as a cause of interstitial nephritis, it certainly does not exonerate other drugs taken concurrently. A condition gains an often unjustified "respectability" when a name is given to it, and once the name "phenacetin nephritis" was established, everyone felt much better, but the incentive to look for other causes seemed to be lost. The present situation is far from satisfactory. There is substantial epidemiological evidence to support the theory that abuse of analgesics containing phenacetin *and other drugs* can, in some instances, cause renal damage. It has been argued that patients with chronic bacterial pyelonephritis are likely to suffer from headaches and therefore to abuse analgesics, and that the frequent association between pyelonephritis and analgesic abuse is therefore coincidental (Reubi, 1958, and others). This is unlikely to explain all cases because some do not have clinical evidence of urinary tract infection during their illness, and analgesic abuse has preceded any renal or other

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symptoms, including headaches. In addition, identical renal lesions have occurred in patients without headaches abusing analgesics because of arthritis (Jacobs, 1964; Tan, Rabbino & Hopper, 1964; Beales, 1965; Kennedy, 1965, and many others) or to increase performance at work (Grimlund, 1963).

The association between renal damage and abuse of analgesics is obscured and complicated by the following facts which must be satisfactorily explained by any acceptable theory of the mechanism of toxic action.

1. Extensive studies in animals treated with phenacetin have failed in general to induce renal lesions comparable to those seen in man. This work has recently been reviewed by Studer (1965).

2. Many persons abusing analgesics (including mixtures containing phenacetin) do not seem to suffer renal damage.

3. Many patients with renal impairment associated with abuse of analgesics have had evidence of pyelonephritis. The relationship between urinary tract infection and analgesic nephritis is not clear. Further complication arises from the difficulty in histological distinction between chronic pyelonephritis and analgesic nephritis.

4. With the exception of one isolated report (Nordenfelt & Ringertz, 1961), females have been affected more frequently than males. This is also true in chronic pyelonephritis, but inadequate information is available to indicate the sex ratio of analgesic abuse itself.

5. There are geographical inconsistencies. The reported incidence of analgesic nephritis has been much greater in Scandinavia and Switzerland than in some other countries despite apparently similar overall national consumption of phenacetin (Ross, 1962).

The incidence of "analgesic nephritis" would appear to be low in this country, and the first case was not published until 1964 (Sanerkin & Weaver). However, a total of nine cases has now been recorded (Sanerkin, 1964; Jacobs, 1964; Brown & Pell-Ilderton, 1964; Beales, 1965; Kennedy, 1965), and I myself have already seen 27 cases in Aberdeen including one case of interstitial nephritis in a patient treated with *p*-aminosalicylic acid for tuberculosis. Together with a further group of patients whose analgesic histories have not yet been established, the total number may exceed 40. The true incidence may therefore be high.

p-Chloracetanilide, a usual contaminant of phenacetin, has been suspected of nephrotoxicity. Harvald, Valdorf-Hansen & Nielsen (1960) found that the administration of phenacetin containing 0·13% and 0·30% of *p*-chloracetanilide to patients with advanced renal damage caused a greater increase in the urinary excretion of red blood cells as measured by Addis counts than when pure phenacetin was given. On the other hand, a significant increase in "leucocyte" excretion occurred only with the smaller dose of contaminating *p*-chloracetanilide and the validity of these findings has been questioned (Kup, 1960).

Surprisingly little work seems to have been done to follow this lead and it was not until last year that Schnitzer, Smith & Golden (1965) published

their work on the chronic toxicity of *p*-chloracetanilide in rats. They found no evidence of renal toxicity in a 22 week study, but did observe profound effects on the blood forming tissues. Although this question must still remain open, it seems unlikely that analgesic nephritis is caused by this compound, but it may contribute to the haemolytic anaemia often encountered.

Perhaps we should think about this problem in a different way. *If* phenacetin is the sole nephrotoxic agent, then it seems clear that it can affect only a minority of persons abusing it. To ascertain the mechanisms of toxicity thus becomes more difficult, as it is not unreasonable to suppose that only a minority of experimental animals treated with phenacetin would develop renal lesions. The study of individual animals rather than groups might therefore be more fruitful, but practical considerations limit this approach. There is also the possibility that phenacetin is only nephrotoxic in the presence of other drugs, and further studies might be worth while using more drug combinations. If other drugs taken together with phenacetin are primarily responsible for the renal damage (as for instance the anti-inflammatory drugs or caffeine), some of the difficulties in accepting phenacetin as a cause of analgesic nephritis might be explained.

RECENT FINDINGS

The assumption has been made that if a drug or its metabolites is toxic and causes tubular damage, cell death may occur and result in an increase in the number of *renal tubular cells* appearing in the urinary sediment. Since it is difficult to differentiate reliably between renal tubular cells and leucocytes, these cells are usually counted together as "non-squamous white cells". The large spontaneous fluctuation in the output of leucocytes (Prescott, 1966) therefore makes it difficult to show any but gross changes in the excretion of renal tubular cells. For this reason the Addis count is a relatively crude and insensitive method. By the use of the diaminofluorene-peroxide-phloxine method (Prescott & Brodie, 1964) it is possible to stain these cells differentially and simply, so that small changes in the excretion of renal tubular cells can be demonstrated.

Groups of healthy volunteers (5 male, 5 female) were given the following drugs orally in four divided daily doses: acetylsalicylic acid (aspirin) 3·6 g, phenacetin 3·6 g, A.P.C. (aspirin 1·8 g, phenacetin 1·8 g, caffeine citrate 1·2 g), paracetamol (acetaminophen) 3·6 g, caffeine citrate 2·4 g and a placebo. Excretion rates of renal tubular cells, leucocytes and red blood cells were determined during a 5 day control period and again during 5 days of drug administration (Prescott, 1965a).

The changes in the excretion of renal tubular cells are shown for each treatment group in Table 1. The mean control counts for each group shown in the first column are the mean total renal tubular cells excreted between midnight and 4.00 p.m. during the 5 day control period. The corresponding counts during the treatment periods are shown in the next column. A great increase in renal tubular cells occurred in the aspirin group, and moderately large increases were seen in the A.P.C., phenacetin and caffeine groups. Since only a small (but statistically significant)

TABLE 1. MEAN TOTAL RENAL TUBULAR CELL COUNTS

Tablets administered	Control counts (thousands)	Drug counts (thousands)	% Increase	P*
Placebo	5,814	6,153	+6	N.S.
Paracetamol†	5,645	6,286	+11	0·05
Caffeine	6,308	8,660	+37	0·01
Phenacetin	6,064	10,494	+73	0·05
A.P.C.	6,012	11,435	+90	0·01
Aspirin	6,065	57,294	+945	0·01
Mean control count	5,900			
95% confidence interval for control counts	4,845–7,015			

* Two-tailed tests.
† Observations on 21 subjects.

increase took place in the paracetamol group, a further 11 subjects (5 male, 6 female) were given paracetamol, with identical results. Significant increases in the excretion of red blood cells occurred only in the groups receiving aspirin, A.P.C. and caffeine, and none of the drugs appeared to have any significant effect on the excretion of leucocytes.

In this original study, only two of the ten subjects taking phenacetin showed a great increase in renal tubular cells. The response of one of these subjects is shown in Fig. 1. Subsequent studies have resulted in

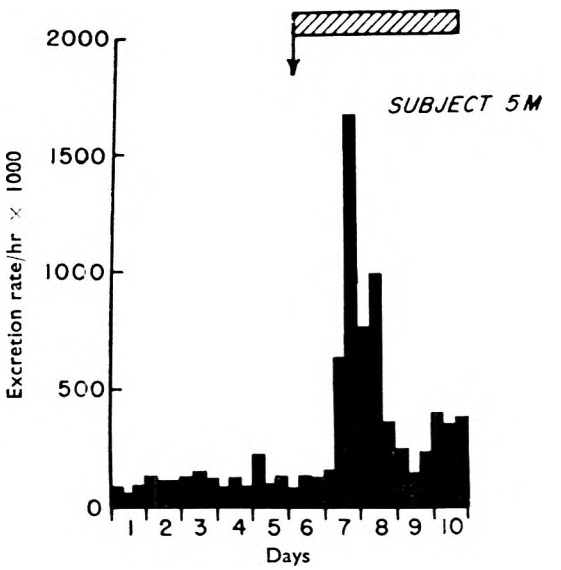

FIG. 1. The effect of phenacetin on the excretion of renal tubular cells in a normal male volunteer. Hatched area: phenacetin 3·6 g/day.

similar marked increases in four of 27 normal persons (15%) given 3·6 g phenacetin daily. In contrast, of 31 persons taking the same dose of paracetamol, only one showed an appreciable increase, and this was much less than the responses seen with phenacetin. In addition, three subjects responding to phenacetin were subsequently challenged with paracetamol.

TABLE 2. RENAL TUBULAR CELL EXCRETION IN 3 SUBJECTS GIVEN PHENACETIN AND PARACETAMOL IN SEPARATE STUDIES

	Phenacetin			Paracetamol		
Subject	Control count (thousands)	Treatment count (thousands)	% Increase	Control count (thousands)	Treatment count (thousands)	% Increase
1	8,792	23,100	−163	8,168	8,352	+ 2
2	6,956	12,372	+ 78	5,336	4,372	−18
3*	8,612	18,592	+116	9,888	15,512	+57

* All subjects received 3·6 g of phenacetin or paracetamol daily for 5 days, except subject 3, who could only tolerate 2·7 g phenacetin.

In two there was no change in cell excretion and in the other, already mentioned above, the increase during the administration of paracetamol was much less than when phenacetin was given. These data are given in Table 2. It is interesting that in this subject, side-effects limited the dose of phenacetin to 2·7 g daily, although 3·6 g paracetamol was easily tolerated. The effect of phenacetin on the renal tubular cell excretion of a patient thought to have analgesic nephritis is shown in Fig. 2. It can be seen that there was a progressive rise in the renal tubular cell counts.

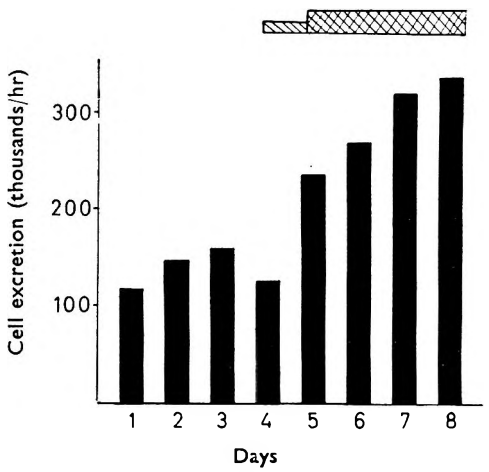

FIG. 2. The effect of phenacetin on renal tubular cell excretion in a patient thought to have "analgesic nephritis". Hatched area: 1·8 g phenacetin daily. Cross-hatched area: 3·6 g/day.

METABOLITES OF PHENACETIN

Since phenacetin is rapidly and extensively metabolised to paracetamol (Brodie & Axelrod, 1949; Welch, Conney & Burns, 1966), this relative lack of effect with paracetamol is of great interest and suggests that either phenacetin itself, or metabolites other than paracetamol, are responsible for this effect. Paracetamol, *p*-phenetidine, 2-hydroxyphenacetin, *S*-(1-acetamido-4-hydroxyphenyl)cysteine and other amines have been identified as metabolites of phenacetin in man (Brodie & Axel-

rod, 1949; Burns & Conney, 1964; Jagenburg & Toczko, 1964; Klutch, Harfenist & Conney, 1966) (Fig. 3), but apart from paracetamol little is known of the renal toxicology of these or other unknown metabolites.

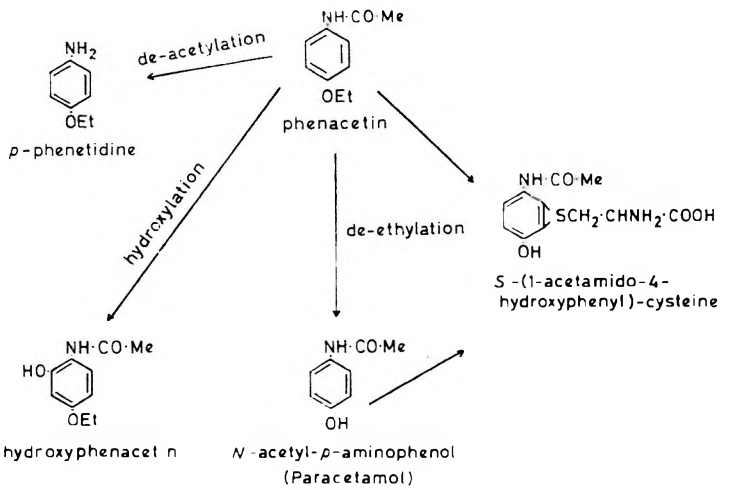

FIG. 3. The metabolism of phenacetin in man.

Preliminary studies (Prescott & Conney, unpublished) have shown no difference in the maximum plasma concentration or half-life of phenacetin and paracetamol after oral administration of phenacetin in subjects showing a marked rise in renal tubular cell excretion compared with those who did not. In nine subjects the mean plasma half-life of phenacetin was 67 min, with a range of 50–90 min. Two subjects with renal tubular cell responses to phenacetin had plasma half-lives of phenacetin of 60 and 70 min. The studies were repeated after drug treatment for 5 days, and although there was wide variation in the peak plasma concentration of phenacetin in the same individual, the half-life was remarkably constant. The combined data are given in Fig. 4.

These findings suggest that only a minority of normal adults is susceptible to the acute renal effects of phenacetin. This effect seems unrelated to the plasma concentrations or half-life of phenacetin, and therefore is unlikely to be due to variations in absorption or to a threshold effect. The possibility that this variation in susceptibility is due to some other metabolic differences, such as the formation of toxic amine metabolites, merits consideration. Although paracetamol is extensively conjugated, de-acetylation and re-acetylation occur in several species and amine metabolites can be formed; in the cat they may account for more than 10% of the administered dose (Welch, Conney & Burns, unpublished). It must be stressed however that although paracetamol apparently does not have a marked nephrotoxic effect in man, it cannot be assumed that it lacks long term toxicity. Caution must also be exercised generally in relating the acute drug effects under discussion to those occurring clinically

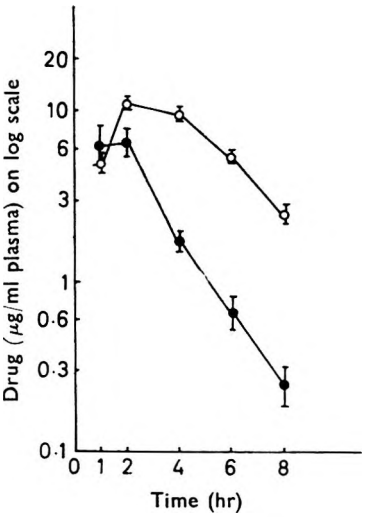

FIG. 4. The mean plasma levels of phenacetin (●—●) and paracetamol (○—○) in 9 normal individuals after 1·8 g of phenacetin by mouth.

following chronic administration. That only a minority of persons is susceptible to the acute renal effects of phenacetin is however in agreement with clinical observations and the possibility of a genetically determined abnormality of phenacetin metabolism is raised. The cell excretion data described are consistent with polymorphism in the renal response to phenacetin (Price-Evans, D., personal communication), and there have been reports of several cases of analgesic nephritis occurring in the same family (Poli, 1955; Ask-Upmark, 1960). One remarkable family history was described by Grimlund (1963) in which six of 11 siblings had analgesic nephritis; three died in uraemia, and several had gastric or duodenal ulceration.

SALICYLATES

Salicylates have long been known to cause renal impairment, and there have been clinical reports of haematuria, proteinuria, increased cells in the urinary sediment, azotaemia, impaired phenolsulphonphthalein excretion, fluid and salt retention, oedema, aminoaciduria, oliguria, anuria, acute tubular necrosis, and papillary necrosis following salicylate administration (see review by Hanzlik, 1927; Lipman, Krasnoff & Schless, 1949; Locket, 1957; Campbell & MacLaurin, 1958; Granville-Grossman & Sergeant, 1960; Harvald, 1963; Scott, Denman & Dorling, 1963; Ben-Ishay, 1964). Tubular atrophy and dilation, interstitial tissue proliferation, decreased resistance to infection, and papillary necrosis have been produced in rats treated with aspirin (Clausen, 1962, 1964; Fellers, Pradilla & Craig, 1965). It is surprising therefore that more attention has not been paid to the renal effects of salicylates. Scott & others (1963) drew attention to the effect of salicylates on the exfoliation

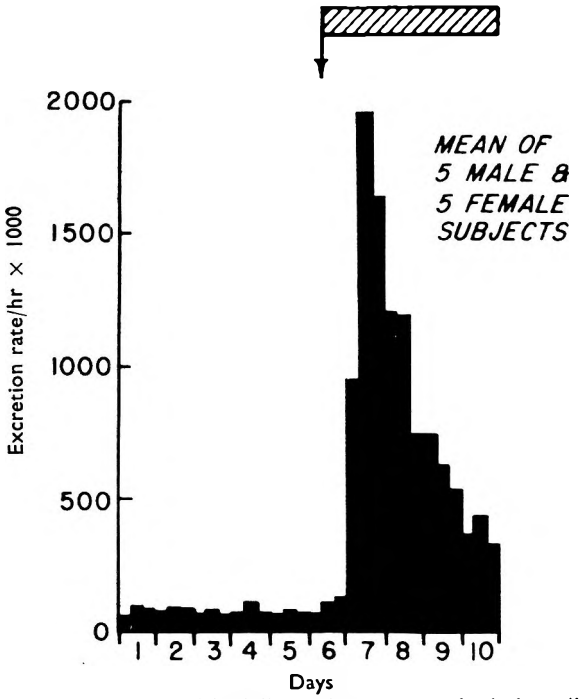

FIG. 5. The effect of 3·6 g aspirin daily on the mean renal tubular cell excretion rates of 10 normal volunteers. Hatched area: aspirin 3·6 g/day.

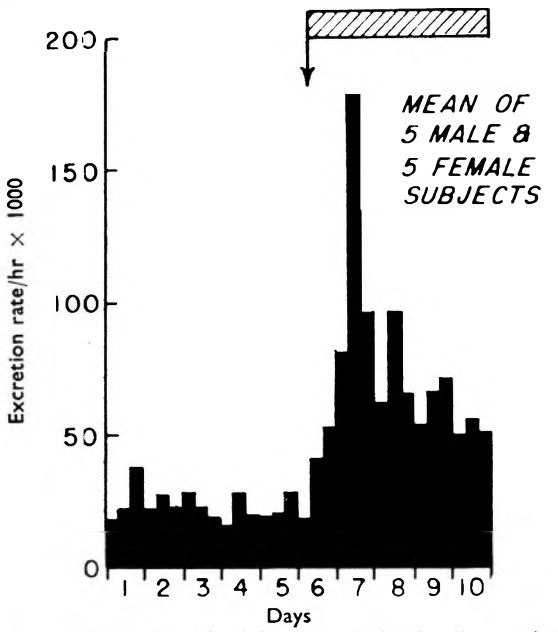

FIG. 6. The effect of 3·6 g aspirin daily on red blood cell excretion in 10 normal subjects. Hatched area: aspirin 3·6 g/day.

of renal tubular cells, and in my recent work, I found that a dramatic increase in the renal tubular cell excretion took place in every subject receiving aspirin. The mean increase in renal tubular and red blood cells is shown in Figs 5 and 6. The increase in both cell types was much greater in females than in males, and this is shown in Figs 7 and 8, which show the individual changes in cell excretion expressed as a percentage of the control counts.

Scott & others (1963) felt that the increased cell excretion was only a transient effect and therefore was unlikely to cause renal drug damage on

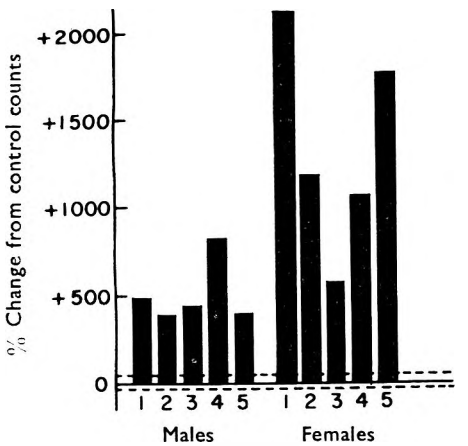

FIG. 7. Individual changes in renal tubular cell excretion in subjects given aspirin 3·6 g daily for 5 days.

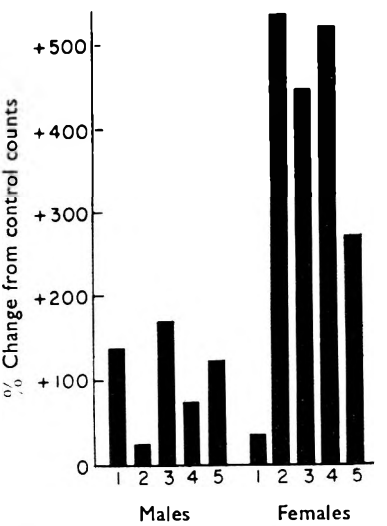

FIG. 8. Individual changes in red blood cell excretion in subjects given aspirin 3·6 g daily for 5 days.

chronic administration. To investigate this further, five subjects (four male, one female) were given 3·6 g aspirin daily for 3 weeks and the excretion of renal tubular cells and red blood cells during this period was compared with the control periods one week before and one week after treatment. The effect on renal tubular cell excretion is shown in Fig. 9

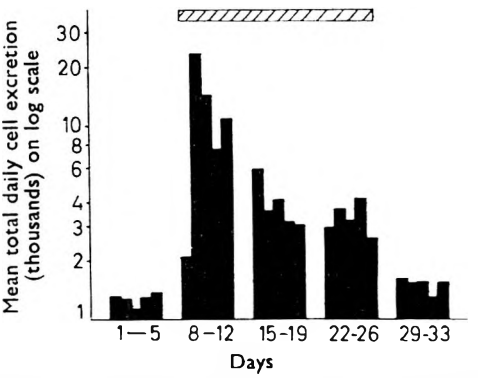

FIG. 9. The effect of aspirin (3·6 g daily for 3 weeks) on the mean excretion of renal tubular cells in 5 subjects (4 male, 1 female.) Hatched area: aspirin 3·6 g/day.

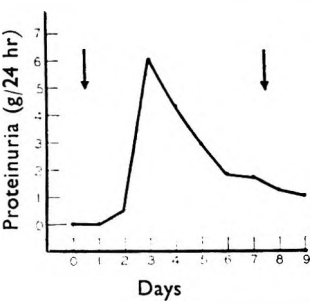

FIG. 10. Mean values for a group of 29 patients with early syphilis receiving bacitracin (400 units/kg) every 5 hr for seven days. Arrows represent beginning and end of treatment.

and it can be seen that although the maximum increase occurred during the first week, the counts were still well above the control levels at the end of the third week of treatment. This phenomenon is not restricted to the salicylates, and an identical response to caffeine was described by Vinci (1914). Renal damage produced by bacitracin results in a rise in urinary cell excretion, proteinuria and impaired renal function (Miller, McDonald & Shock, 1950). In Fig. 10 it can be seen that the maximum urinary protein loss occurs on the second day of treatment, and subsequently falls despite continued drug administration (cf. Fig. 5).

A high incidence of peptic ulceration has been noted in patients with renal damage associated with analgesic abuse. Salicylates are known to cause gastric ulceration and salicylate-induced gastrointestinal bleeding may contribute to the anaemia. Furthermore the unexplained acidosis

reported in many cases of analgesic nephritis could be due to the ingestion of large amounts of salicylates in the presence of renal failure. Phenacetin does not cause acidosis or peptic ulceration.

Patients with rheumatoid arthritis often consume large quantities of analgesics, especially salicylates. Clausen & Pedersen (1961) found that 23% of a series of 80 patients with rheumatoid arthritis had post-mortem evidence of papillary necrosis, and Brun, Olsen, Raaschou & Sørensen (1965) found that nine out of 32 patients with rheumatoid arthritis had chronic interstitial nephritis by renal biopsy. Sørensen (1963) and Allander, Bucht, Lövgren & Wehle (1963), were unable to find any correlation between renal impairment and phenacetin consumption in such patients. Sørensen, however, showed that renal function (as measured by endogenous creatinine clearance, which does not measure tubular function) was progressively impaired with increasing severity of the rheumatoid arthritis. Since those patients with the more advanced arthritis were likely to have received more drugs than those with milder disease, it cannot be stated yet whether the renal lesions (including interstitial nephritis and papillary necrosis) found in these patients are due to the underlying arthritis or induced by analgesic or other drugs. There have been no studies reported in which an attempt has been made to correlate renal damage with salicylate consumption in patients with rheumatoid arthritis.

Apart from the renal effects of the salicylates, other serious toxic effects include gastrointestinal bleeding, ulceration and perforation (Douthwaite & Lintott, 1938; Alvarez & Summerskill, 1958; Duggan, 1965, and others), exfoliation of cells from the gastric mucosa (Croft, 1963), "allergic" phenomena (Locket, 1957; Viguie & Gardies, 1963), aplastic anaemia (Erslev & Wintrobe, 1962; Snijder, Wijnja & Nieweg, 1965) and agranulocytosis (Pretty, Gosselin, Colpron & Long, 1965). In view of the tremendous consumption of salicylates, it is surprising that serious toxic effects are not encountered more frequently.

ANTIPYRINE GROUP AND OTHER ANTI-INFLAMMATORY AGENTS

As salicylates have been largely replaced by the antipyrine group in analgesic mixtures in many European countries with a high incidence of analgesic nephritis, it has been argued that salicylates cannot cause chronic interstitial nephritis. This argument is not valid if the anti-inflammatory drugs which replace salicylates in these mixtures have similar nephrotoxic properties. This indeed may be the case. Antipyrine, aminopyrine, and related pyrazolones are commonly substituted, and we find that the same formidable list of nephrotoxic effects as seen with salicylates has in fact been encountered with the antipyrine group (Lotze, 1934; Aosima, 1940; Axelsson, 1958; Fazekas, Fazekas & Bertok, 1960; Eknoyan & Matson, 1964). The related anti-inflammatory drug phenylbutazone has similar nephrotoxic effects (Lipsett & Goldman, 1954; Steinbrocker, Neustadt & Ehrlich, 1954; Weisman & Bloom, 1955; Bruck, Fearnley, Meanock & Patley, 1954) in addition to other unpleasant effects such as agranulocytosis (see Gsell, 1954). The reported renal

effects of the salicylates, antipyrine and aminopyrine, and phenylbutazone include haematuria, increased cells and casts in sediment, proteinuria, sodium chloride, fluid and nitrogen retention, oliguria, anuria, tubular necrosis and except for phenylbutazone, papillary necrosis. These drugs share many corticosteroid-like actions, and like the steroids, salicylates and phenylbutazone can cause gastric ulceration and fluid retention. Indomethacin, a newer and chemically dissimilar anti-inflammatory drug also seems to cause peptic ulceration and fluid retention, and flufenamic acid (and to a lesser extent mefenamic acid) causes tubular damage and papillary necrosis in animals. It is interesting that many anti-inflammatory agents have been shown to displace corticosteroids from plasma proteins (Maickel, Miller & Brodie, 1965) but it has not been established that this effect is related to anti-inflammatory or toxic effects. Anti-inflammatory action could be the link between toxic renal damage and subsequent pyelonephritis if susceptibility to infection is reduced—an attractive but as yet unproven theory.

CAFFEINE

Finally it is necessary to consider caffeine. This drug has been present in almost all the analgesic mixtures associated with interstitial nephritis and papillary necrosis, and epidemiologically should be as suspect as phenacetin. It is a gastric irritant, capable of producing gastric ulceration (Pfeiffer & Gass, 1962) and there is both clinical and experimental evidence to indicate nephrotoxicity (Vinci, 1914; Wendt, 1938; Boyd, 1959; Boyd, Dolman, Knight & Sheppard, 1965; Prescott, 1965b) Little attention seems to have been given to the possibility that caffeine, abused in analgesic mixtures, or in beverages could have any part in the aetiology of analgesic nephritis. In the present study most persons receiving caffeine showed a rise in renal tubular and red blood cell excretion, although there was no appreciable sex difference in response. Caffeine, like the salicylates, has teratogenic effects (Nishimura & Nakai, 1960; McColl, Globus & Robinson, 1965; Trasler, 1965), and the stimulating effect on the central nervous system would seem a likely explanation of habituation to analgesic mixtures.

SUMMARY

In summary the situation is very confused, but certain points emerge. *If* phenacetin is the only drug responsible for analgesic nephritis, then apparently only a minority of persons abusing it is affected. The evidence discussed supports this hypothesis and suggests that this susceptibility has a metabolic basis. Many more sophisticated experiments will be necessary to establish and demonstrate the precise mechanisms of toxicity. Alternative or additional nephrotoxicity may be contributed by the salicylates, the antipyrine group of drugs, caffeine, and perhaps other drugs.

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Discussion

Dr. A. W. S. Sørensen. In five investigations, I have examined the relationship between consumption of analgesic agents and changes in the kidneys. In a consecutive evaluation of 3 days creatinine clearance in 790 patients, 244 of whom had rheumatoid arthritis, no relation between clearance and consumption of analgesics was found, especially in the groups with heavy consumption where such changes as chronic interstitial nephritis and chronic pyelonephritis were suspected. In 191 women, 50 of whom had rheumatoid arthritis and the remainder other diseases, but all without previous kidney disease or urinary tract complaints, there was no relation between the specific gravity of the urine and the intake of analgesics. In kidney biopsies from 32 patients with rheumatoid arthritis no relation was found between intake of analgesics and the histological picture. In a consecutive controlled investigation, over one year, of the incidence of bacteriuria (more than 100,000 organisms per ml), among 126 consumers of analgesics, compared with the same number who had not taken them, we found the same incidence of 20% in both groups. In a consecutive controlled X-ray study of 1,000 patients from all departments of Copenhagen Commune Hospital, in 1963–64, I found 167 patients (about 17%) with a chronic analgesic consumption. Among these, 8% had papillary necrosis compared with 5% among the other 833 patients. These figures are high and must be reduced because there were cases with obstructions in the urinary tract.

As a clinician I think this problem now requires a quantitative dimension. I should like to know the prevalence of so-called chronic pyelonephritis,

as we already know the prevalence of asymptomatic bacteriuria and kidney diseases of all kinds among consumers in different geographical regions, without regard to any causality between analgesic agents and renal diseases. I think such a comparison will give the wanted dimension. May I ask Dr. Prescott if he is able to exclude that the increasing desquamation of cells in the tubules in his investigations is a chemical acceleration of a normal phenomenon which is self-limiting? Take the work of Scott and his colleagues (1963)* and the Fig. 9 in the present paper, for instance. Desquamated cells will always be found in the lumen of tubules in a so-called normal biopsy. I agree that these studies of short duration—days or weeks—cannot tell us anything about what will happen in the kidneys during 10, 20 or 30 years consumption of analgesic agents.

Dr. A. Kennedy. My colleague, Dr. Davies, and I have sought answers to the questions: does necrosis in the kidney necessarily produce renal tubular cells in the urine? Does an increase in cells in the urine necessarily indicate necrosis in the kidney? Is there any quantitative relation between these two conditions, and are they directly proportional to one another? We have used known nephrotoxic substances in experimental animals so that we could examine the urine, kill the animals and then examine the kidneys. We have used mercuric chloride which is known to damage the proximal convoluted tubules. A single dose of about 1 mg/kg of mercuric chloride given to rats produced, within 28–48 hr, about a hundredfold increase in the output of cells in the urine, and histologically there was a minimal necrosis in the proximal convoluted tubules. We have also used ethylenimine which causes necrosis of the papillae, a lesion not unlike that described in analgesic nephritis in man. When given to a rat in a dose which is not immediately fatal (it is also a neurotoxic) the papillae can be destroyed and a lesion, histologically very much more severe than the mercury induced lesions of the cortex, is produced. But this produces only about a 10- or 20-fold increase in the output of cells. Not only is the cell excretion not directly proportional to the degree of damage, but it may also depend on the site in the kidney which is damaged by the agent. We would agree that those toxic agents causing necrosis will increase the number of cells in the urine, but we have not yet decided whether it is possible to produce a "cell-uria" in the absence of necrosis. One suggestion why the urinary cell count rises and then falls is that in some kidneys aspirin or phenacetin or a similar drug knocks out older cells which are then desquamated, leaving the younger and more resistant cells behind, so that the cell count gradually falls to normal again.

Is the same sort of cell excreted in response to aspirin that is excreted in response to phenacetin?

Dr. N. G. Sanerkin. The desquamation of renal tubular epithelial cells after the administration of aspirin, phenacetin, or other drugs has no real relevance to the renal lesions occurring in prolonged analgesic abuse. I believe that in such persons the primary lesion is renal papillary or medullary necrosis and that the renal parenchymal contraction ("chronic

* See p. 353 for references.

interstitial nephritis") is merely secondary to the medullary changes (Sanerkin, 1966). Renal medullary necrosis causes disruption of important medullary structures, including Henle's tubules, interrupting nephronal function, and the vasa recta, producing ischaemic damage in the cortex, mainly by interfering with the venous return; it also predisposes the kidney to superimposed infection. As a result of these processes the related cortex undergoes atrophy and fibrosis. An identical view has just been independently expressed by Dawborn, Fairley, Kincaid-Smith & King (1966) who conclude that most of the changes of so-called chronic interstitial nephritis are a direct consequence of renal papillary necrosis, and suggest that the term "chronic interstitial nephritis" should be discarded in cases of analgesic nephropathy. Dr. Kennedy finds that a chemical like carbon tetrachloride, which is toxic to the proximal renal tubular epithelium, causes heavy desquamation of tubular epithelial cells, whereas vinylamine, which produces renal papillary necrosis, causes no significant epithelial desquamation. Obviously the solution to the problem of chronic analgesic nephropathy must be sought not in the immediate effect of suspected drugs on the renal tubular epithelium but in their long-term effect on the renal medulla itself.

Dr. J. T. Scott. I should like to take up this question of aspirin and kidney damage. I am not so concerned about phenacetin, but aspirin is a very useful analgesic and anti-inflammatory drug and we should be careful before we incriminate it in this respect. Now as we showed a few years ago (Scott, Denman & Dorling, 1963), anyone taking aspirin passes a large number of tubular cells in the urine (Fig. 1). This is a

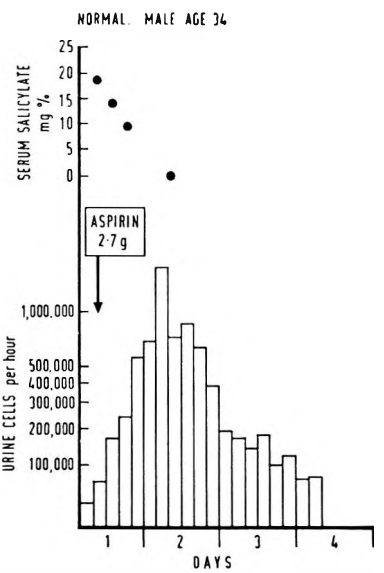

FIG. 1. *Effect of a single dose of 2·7 g aspirin on excretion of renal tubular cells in urine in a normal male.

*Figs 1–4 of this contribution are reproduced from Scott & others (1963), *Lancet*, **1**, 344–348, by permission of the Editor.

universal finding, the only exception being if the subject has taken aspirin previously in recent weeks. A second dose of aspirin taken a few days after the first produces no further exfoliation of cells, but as the time interval between the two doses is lengthened a response is seen to the second dose, though this is still diminished, at least for a month or so (Fig. 2). The shedding of tubular cells is more or less transient and if the administration of the drug is continued the cell count falls to normal levels (Fig. 3). We studied two patients continuously for several weeks, and during the last week the mean cell count in both of them was no higher

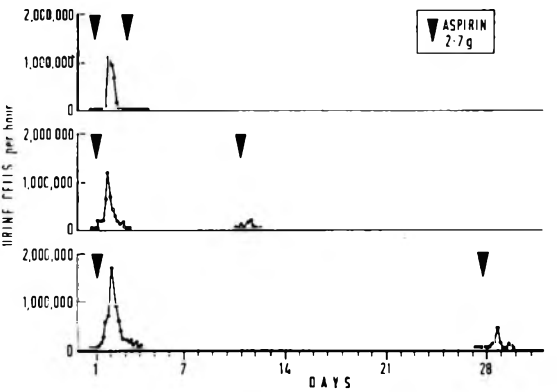

FIG. 2. Effect on excretion of renal tubular cells in urine of single doses of aspirin 2·7 g repeated at different intervals in three subjects.

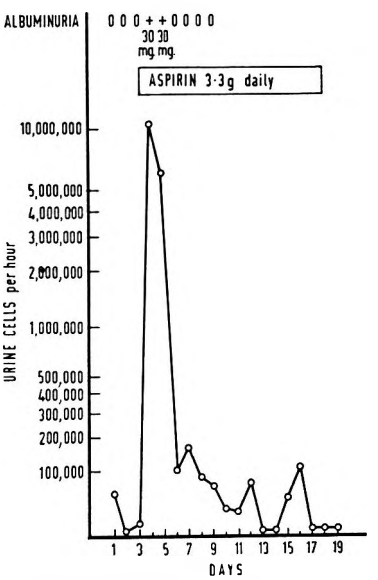

FIG. 3. Effect on excretion of renal tubular cells in urine of continued salicylate in a boy of 11 with rheumatic fever.

than that before aspirin was commenced (Fig. 4). Aspirin celluria is mostly a phenomenon of the early weeks of treatment. It seems likely that aspirin causes the premature desquamation of tubular cells which have attained a certain degree of maturity. Younger cells are not shed and so it is only after several weeks' abstinence from salicylates, during which the cells are permitted to grow, that readministration produced a further celluria.

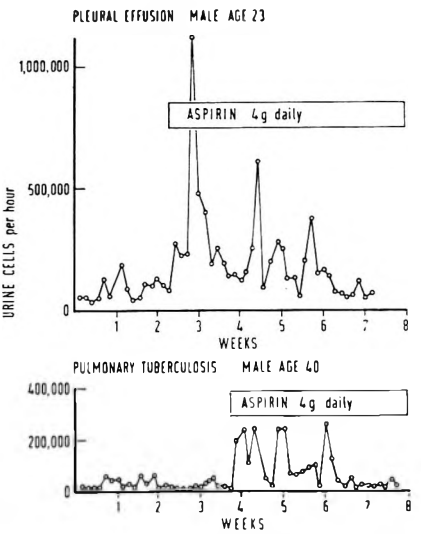

FIG. 4. Daily urine cell-counts in two patients, continued for several weeks of aspirin treatment.

There is really nothing to indicate that this acute tubular cell desquamation—which happens to everyone—has anything to do with chronic renal damage. As animal evidence is so far unsatisfactory we come back to clinical experience. Now Dr. Prescott makes the point that kidney damage has never been reported when phenacetin has been taken alone. But I have never heard of anybody prescribing phenacetin alone. Although it is available as the plain tablet it is nearly always administered in a compound analgesic mixture. This does not apply to aspirin, which is frequently given alone, and in enormous quantities both in this and other countries. As far as I know, however, with the exception of a single individual in a large survey of patients with renal papillary necrosis (Harvald, 1963), there have been no reports of such a condition following the long-term use of aspirin. I do not think the present evidence implicates salicylates as a cause of chronic nephrotoxicity.

Dr. L. F. Prescott. I think Dr. Sørensen's query about the significance of the rise in renal tubular cell excretion has been partly answered by Dr. Kennedy. If a nephrotoxic drug is given, a striking increase in renal tubular cell output occurs, and this can be correlated with histological changes in the kidney. The rise in renal tubular cell count is a more

sensitive indicator of toxic tubular damage than direct histological examination—a small dose of drug may cause increased cell excretion but no obvious histological abnormalities, while larger doses affect more cells and frank tubular necrosis may occur. Increased renal tubular cell excretion is irrefutable evidence of kidney injury (Balazs, Hatch, Zawidzka & Grice, 1963).

When animals (and man) are exposed continually to small doses of acutely nephrotoxic agents such as the heavy metals cadmium and lead, fibrotic lesions similar to those occurring in patients abusing analgesics are seen, and if there has been chronic exposure to *any* substance toxic to the renal tubules I do not consider it unreasonable to propose that this might eventually result in tubular degeneration, atrophy and fibrosis. With regard to the point raised by Dr. Kennedy, the renal tubular cells appearing in the urine following treatment with phenacetin were indistinguishable from those seen with aspirin.

I would like to point out that the 27 cases of analgesic nephritis that I have encountered in Aberdeen have corresponded closely to those reported from Scandinavia and Switzerland in that they had disease clinically similar to chronic pyelonephritis, sometimes with infection and papillary necrosis, sometimes without, often with a history of peptic ulceration or gastrointestinal bleeding and refractory anaemia.

I agree with Dr. Sanerkin concerning the medullary changes. These have been most marked in the preparations that I have examined. In the cortex the appearances may be almost normal with very little fibrous interstitial tissue, while towards the papillae this increases markedly and is often associated with sclerotic papillary necrosis.

With regard to the role of salicylates, I agree that aspirin cannot be blamed solely on the basis of these results. On the other hand, there is overwhelming evidence in the literature that salicylates can produce severe renal damage, and this does not all refer to acute administration of the drug. Can we really ignore this?

Dr. D. V. Parke. I have seen a record which suggests that the toxic side-effects of phenacetin may be genetically determined. The case was of a young girl in Zurich who had cyanosis. The metabolites in the urine of this patient were examined and the 3-hydroxy metabolite, not the 2-hydroxy metabolite, of phenacetin was found—the first time this has been recorded. 3-Hydroxy-4-methoxyaniline was also found, and this substance could give rise to 3,4-dihydroxyaniline (4-aminocatechol) which is an extremely toxic substance. I have fed this substance to dogs at 10 mg/kg and they all died with severe haematuria. Maybe deacetylation of these dihydroxy products could occur in the kidney giving rise to the toxic dihydroxyaniline products. They would probably not be found in the urine because they combine avidly with the organelles of the kidney tissues. The clinical symptoms of this girl were exacerbated, and the 3-hydroxy metabolite in the urine was increased, by treatment with phenobarbitone, which is known to increase the hydroxylation of a number of drugs. This case could be a genetic abnormality, giving rise to an aberration in metabolism of phenacetin.

DISCUSSION

Mr. T. L. Hardy. We have felt for some time that the antipyrine group of drugs might bear at least part of the blame for many of the renal lesions seen. Many Scandinavian and other continental reports, for example that of Horisberger, have recorded case histories and studies dealing with "phenacetin nephritis". In most of these reports phenazone or a phenazone derivative has been included in the formulations used, as in Saridone. These derivatives may be present in equal proportions to phenacetin, or phenacetin may be absent as in Kafa. Kafa and Saridone were reported by Horisberger and his colleagues (1958) as the most frequently taken preparations noted in his study. Again, Grimlund (1965) repeatedly discusses the effects of phenacetin consumption, in his study of the population of Husqvarna, in Sweden, in subjects taking an equal quantity of phenazone. Recently, during comparative studies of analgesics, including the antipyrine group, we have noted a large and highly significant elevation in the number of renal tubular cells in the urine of rats after the administration of phenazone. This has been accompanied by a proteinuria and in some instances a glycosuria. The cells were identical in morphology to those noted after the administration of known nephrotoxics.

Professor A. H. Beckett. Considering the metabolites of phenacetin, which are implicated in its toxic effects, if it is phenacetin that causes the trouble, and deacetylation is one of the metabolic pathways, surely *p*-phenetidine should be considered as a subject for detailed examination. It only needs the urine of some individuals to be more alkaline than others for reabsorption of this particular metabolite.

Dr. L. F. Prescott. The formation of the 3-hydroxy metabolite of phenacetin has been suspected, although I do not think it has previously been demonstrated in man. De-acetylation of these hydroxylated metabolites may occur, giving rise to 2-hydroxy-4-ethoxyaniline and 3-hydroxy-4-ethoxyaniline, while subsequent dealkylation could yield 4-aminoresorcinol and 4-aminocatechol respectively. The toxicity of these metabolites may therefore be very relevant. Since many patients abusing analgesics also habitually take barbiturates (which are known to increase the activity of several drug metabolising enzymes), it is significant that increased formation of toxic metabolites or their precursors could be demonstrated when the patient described by Dr. Parke was treated with phenobarbitone. Pletscher, Studer & Miescher (1958) have shown that *p*-phenetidine produces a greater reduction in erythrocyte survival time in rabbits than either phenacetin or paracetamol, but little is known of the effects on the kidney.

There would seem to be a convincing epidemiological case against the antipyrine group of drugs on the basis of the high reported incidence of analgesic nephritis in Sweden, Denmark and Switzerland—countries where the consumption of antipyrine is particularly high. In addition, Axelsson (1958) has described papillary necrosis following the abuse of antipyrine so that Mr. Hardy's findings are most interesting.

Dr. A. W. S. Sorensen. On the Continent we have examined the problems of nephrotoxicity of analgesics and a few workers have looked

at the prevalence of these kidney diseases, which I still think to be hypothetical. In some studies, the incidence has been found to be much less than 7%, which is the incidence of renal diseases suspected as causing toxic effects among consumers of analgesics. In our own study of hospital patients we found an incidence of papillary necrosis of 8% among consumers, so the 40 cases of suspect toxic nephritis mentioned by Dr. Prescott I believe to be drawn from at least a thousand consumers in an epidemiological study. It is not possible to prove how many of these 40 cases have had a silent chronic pyelonephritis, a phenomenon forgotten by many doctors. Most of the literature on that problem has described urinary tract infections in from 50–85% among consumers of analgesics with renal disease, so although it is often postulated that these renal diseases are actually cases of toxic nephritis, I think we are dealing with bacterial chronic nephritis rather than a toxic manifestation. The prevalence of chronic asymptomatic bacteriuria in different populations is about 2% in men and 4% in women.

To sum up, I think the prevalence of chronic pyelonephritis and so-called analgesic nephritis has the same dimension and may be the same degree of prevalence as asymptomatic chronic bacteriuria, and that there must be a substantial overlapping between these conditions.

Dr. D. A. Price Evans. Dr. Prescott has presented data that four out of 27 patients were high tubular cell shedders. Before accepting that this might be a pharmacogenetic polymorphism, could not the patients be latent pyelonephritics, in which the drug is acting like a steroid or a pyrogen provocative test? Has Dr. Prescott studied the relatives of high cell shedders to see if this is indeed a genetically determined trait?

Professor O. L. Wade. What was the past experience of these shedders of large numbers of cells in relation to their past phenacetin taking? It seems from the data presented that there are some people who are more liable than others to serious damage when they take drugs, perhaps this only becomes manifest when they have taken drugs, and perhaps methods could be devised for picking out which individuals in our community are liable to this.

Dr. D. I. Macdougall. Only some of those exposed to chronic ingestion of large doses of phenacetin develop renal damage and it seems to be agreed that differences in the metabolic pathway for the drug are the likely explanation of this. It has been assumed that one of its metabolic products is directly nephrotoxic. Is it possible that the primary effect of this metabolite is rather the formation of methaemoglobin (and sulphaemoglobin) in the blood? Resultant impairment of oxygen availability in the renal papilla could account for the necrotising papillitis. All of the three patients I have seen with necrotising renal papillitis and history of phenacetin abuse had the characteristic cyanosis of the methaemoglobinaemia which this drug can produce.

Dr. D. J. Davies. Papillary necrosis during the past 10–15 years has shown striking alterations in reported incidence. It is not really a single disease but may be found in a number of conditions. The number of cases has increased by between 10- and 100-fold. Furthermore, there has

been an alteration in the clinical and the pathological pictures in these cases. The classical lesion which occurs in diabetics with urinary infection usually begins as a wedge of infarction at the base of the medulla, but in patients with presumed analgesic nephritis the lesions tend to be limited to the distal part of the papilla. In this respect the lesions resemble the toxic lesions produced by ethylenimine.

Until recently there has been little evidence that phenacetin and other analgesics are nephrotoxic to animals. Within the past 2 years two papers have appeared, one from Abrahams and his co-workers (1964), the second one more recently in America (Fordham, Huffines & Welt, 1965) where a significant number of medullary as well as cortical lesions were observed in animals that had been given phenacetin alone, and also a mixture of aspirin, phenacetin and caffeine. Abrahams & others observed that if the rats were given aspirin and phenacetin together in the same proportions as in an A.P.C. tablet, there was a lower incidence of renal damage in the former group. This may suggest that caffeine is nephrotoxic.

A third point that I would like to make is that Dr. Sørensen screened rheumatoid patients for chronic renal damage by the use of endogenous creatinine clearance. This is a measure of glomerular function and in medullary necrosis and possibly other forms of tubular damage this test may remain normal for some time; the earliest evidence of impaired tubular function is shown by impaired regulation of the osmolarity of the urine in a water concentration and dilution test or in response to a test dose of anti-diuretic hormone.

Professor G. Brownlee. Dr. Prescott reminded us of the nephrotoxicity seen with bacitracin, and in particular of the excretion pattern of the cells showing a peak and then a falling off of the number of cells excreted. When Dr. Eileen Short and I described the nephrotoxicity of polymyxin B we were so impressed by the temporary nature of this acute effect in rats that we ventured to speak of a repair process. We found the effect could be prevented by the simultaneous administration of those amino-acids which donated methyl groups, like methionine and methylcystine.

Dr. L. F. Prescott. I cannot give Dr. Sørensen the true incidence of this condition in Aberdeen as I have not yet attempted to establish this. What I do know is that when I started to look for such patients I found them. The combination of peptic ulceration or gastrointestinal bleeding, refractory anaemia and "chronic pyelonephritis" seems virtually diagnostic—every patient that I have encountered so far with this triad has turned out to be a heavy consumer of analgesics.

Dr. Price Evans has raised the possibility that the four subjects responding to phenacetin with a great increase in renal tubular cell excretion in fact had latent pyelonephritis. While this cannot be entirely ruled out, an incidence of four out of 27 healthy adults would be unlikely, especially as two were males. They had no clinical history of renal disease and had normal urinary findings prior to the study. A study of the relatives both of these subjects and also of patients with analgesic nephritis would certainly be very valuable, and might provide evidence for a genetic mechanism.

DISCUSSION

It was incredibly difficult to find young healthy adults for this study who had not taken analgesics for six weeks previously. A person who has not taken analgesics for this period probably does not take analgesics very frequently, so in this respect the population was somewhat selected. I could find no difference in the control cell excretion or in the response to drugs between those subjects who rarely took analgesics and those who took them more frequently. As it happened, all the four subjects responding to phenacetin rarely took analgesics and there does not seem to be any way of predicting the response to phenacetin at present.

The weight of evidence is against methaemoglobin and sulphaemoglobin being the primary nephrotoxic agents. It is difficult to implicate tissue anoxia resulting from the formation of these pigments since patients with chronic anaemia do not have these renal lesions yet presumably also have a relative anoxia. Again, in congenital methaemoglobinaemia renal damage is not seen. Experimentally, methaemoglobin can produce acute tubular damage, but only in the presence of acidosis. Since acidosis can occur with salicylates and also in renal failure this mechanism could be responsible in theory, but would have to be a secondary effect. It could not apply, for instance to patients with normal or only moderately impaired renal function who were not taking salicylates.

As Dr. Davies points out, several workers have recently reported renal lesions in animals treated with phenacetin and A.P.C. mixtures. Nevertheless, it has taken a long time to do so, and there have been many more negative studies than positive. I think that the results of Fordham must be examined critically, because very large doses of phenacetin (up to 3,000 mg/kg/day) were required and the renal lesions were not comparable to those seen clinically.

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The action of analgesic substances on the gastric mucosa

D. N. CROFT

CLINICAL EFFECTS ON THE GUT OF INGESTION OF ASPIRIN, PHENYLBUTAZONE, INDOMETHACIN AND PARACETAMOL

THREE commonly used analgesics, aspirin, phenylbutazone and indomethacin, have undesirable effects on the gastrointestinal tract of man. Aspirin, the most widely used, causes upper abdominal discomfort and exacerbation of peptic ulcer symptoms in one of 20 subjects every time they take it by mouth (Muir, 1963) and French workers claim that there is a correlation between aspirin ingestion and the development of peptic ulcer (Levrat & Lambert, 1960). An association between aspirin and gastrointestinal bleeding has been demonstrated in two ways. Firstly, more patients admitted to hospital with a severe gastrointestinal bleed have been found to have taken aspirin during the preceding few days than patients admitted for other reasons (Alvarez & Summerskill, 1958; Muir & Cossar, 1959; Parry & Wood, 1963). Secondly, most subjects taking repeated doses of aspirin bleed slightly (Stubbé, 1958; Wood, Harvey-Smith & Dixon, 1962) and this may occasionally lead to iron deficiency anaemia (Summerskill & Alvarez, 1958).

Phenylbutazone also has upper gastrointestinal effects. Ten % of subjects taking this drug may develop nausea, vomiting or upper abdominal pain, 1% peptic ulceration and ½% gastrointestinal bleeding (Mauer, 1955). These effects may be largely a function of dosage.

Indomethacin, when first introduced, was in tablet form and caused perforated peptic ulceration and overt and occult gastrointestinal haemorrhage (Wanka, Jones, Wood & Dixon, 1964), particularly when given in large doses (Lövgren & Allander, 1964). On the introduction of powdered indomethacin in gelatin capsules, no serious gastrointestinal effects were found by Wanka & Dixon (1964) or by Hart & Boardman (1965). Of 137 patients with rheumatic disorders treated with indomethacin capsules by Thompson & Percy (1966) only one developed dyspepsia; one, with a previous history of peptic ulceration, had a slight melaena and one, without previous history of peptic ulceration, perforated an acute duodenal ulcer. These authors concluded that the incidence of indomethacin-induced side-effects originating in the gastrointestinal tract was small.

It is gratifying to find one commonly used analgesic, paracetamol, which does not cause abdominal discomfort, or overt or occult gastrointestinal bleeding (Wood & others, 1962; Goulston & Styring, 1964).

The incidence of dyspepsia, peptic ulceration and gastrointestinal bleeding associated with ingestion of these four analgesics has been assessed by studying groups of patients taking them. This approach has

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provided useful data on the incidence of pain, peptic ulcer or haemorrhage, but has been less helpful in explaining the mechanism whereby the drugs cause these undesirable effects. I wish to present evidence that aspirin is an irritant which causes cell exfoliation from the stomach and to suggest that this may be the basic mechanism whereby the aspirin-induced gastrointestinal bleeding lesion is produced.

SALICYLATE-INDUCED OCCULT BLEEDING INTO THE GUT

Occult bleeding into the gut after aspirin ingestion has received much attention recently and provides a tool with which to measure an irritant effect of this analgesic. Of 226 subjects with an apparently normal gastrointestinal tract, Wood and his colleagues found 78% bled more than 2 ml/day (Wood & others, 1962, Croft & Wood, unpublished). The bleeding was measured by the ^{51}Cr-labelled red cell technique and the volume of the blood lost was found to be relatively reproducible in an individual. Most subjects lost 2–5 ml/day and 10% lost more than 10 ml/day.

Apart from effervescent aspirin, which caused less bleeding, soluble and buffered aspirins, whether or not dissolved, caused the same amount of bleeding as plain aspirin tablets (Wood & others, 1962; Wood, 1963). However, these workers found that preparations which prevented release of aspirin in the stomach, by enteric coating or otherwise, significantly reduced blood loss. These data suggest that salicylate-induced occult bleeding is the response of the stomach to some physiological effect of repeated doses of the drug.

What physiological phenomenon can account for this effect? Roth (1963) has reviewed the various hypotheses that have been considered but none adequately explains the occult bleeding data. Mucosal irritants have been studied by Hollander who wrote in 1946 that, in the gut, surface epithelial "desquamation must be considered a normal physiological response to mild irritation" (Hollander, Stein & Lauber, 1946). In 1947, Wolf & Wolff, in their subject with a gastrostomy, found that this was so with irritants such as ethanol, clove oil, copper sulphate, hydrochloric acid and a suspension of mustard in water. Creamer, Shorter & Bamforth (1961) also observed it in the dog small intestine when the mucosa was perfused with physiological solutions which were too warm (40°–45°).

GASTRIC EPITHELIAL CELL TURNOVER AND MEASUREMENT OF CELL LOSS FROM GASTRIC MUCOSA

To appreciate the significance of increased epithelial cell loss after irritants it is necessary to consider the normal turnover of gastric mucosa. Surface epithelial cells are continuously formed by mitoses in the neck of the gastric glands (Bizzozero, 1893). After formation they migrate up the wall of the gastric pits to be extruded in a degenerate form into the lumen of the stomach (Stevens & Leblond, 1953). In man the life span or turnover of gastric surface cells is about two to six days, whereas

that of acid and pepsin secreting cells in the glands is much longer (MacDonald, Trier & Everett, 1964).

A clinical method has been developed for measuring the natural rate of cell loss from human gastric mucosa by estimating deoxyribonucleic acid (DNA) in gastric washings (Croft & Lubran, 1965). As fairly constant amounts of DNA are found in each human somatic cell irrespective of its type (Davidson, Leslie & White, 1951), the DNA content of a cellular specimen is proportional to the number of cells in it. It is in effect a cell count.

In the method, the stomach is emptied of fasting contents and the gastric mucosa is continuously perfused for 45 min with warm (29°–30°) saline. From the DNA content of the aspirate a rate of accumulation of DNA in the stomach is obtained. Under defined conditions in which the patients do not swallow or regurgitate duodenal fluid, the gastric DNA rate is obtained. In the four subjects on whom duplicate tests were made from 5–19 weeks apart, the gastric DNA rate was found to be reproducible within ±10% over a 13-fold range of values (Table 1). Twenty-eight

TABLE 1. REPRODUCIBILITY OF GASTRIC DNA RATE

Case No.	Weeks between tests	Gastric DNA rate (ng atoms DNA-P/min)	
		1st test	2nd test
13	19	6	5
4	11	12	12
11	5	52	42
24	15	73	71

By continuously perfusing human gastric mucosa with saline and estimating DNA in the aspirate, gastric DNA rates were measured in four subjects on two occasions 5 to 19 weeks apart. The DNA values, which are given in ng atoms DNA-P/min, indicated a reproducibility of ±10%. The gastric DNA rate is considered to measure physiological cell loss from gastric mucosa and to be an index of gastric surface epithelial turnover. (Data abstracted from Croft, Pollock & Coghill, 1966.)

subjects have been studied by this technique and the gastric DNA rates indicate that under physiological conditions not only is there a continuous production of surface cells but a continuous loss of them into the gastric lumen (Croft, Pollock & Coghill, 1966). In the steady state these two parameters must be in equilibrium (Stevens & Leblond, 1953).

To determine what aspirin does to this equilibrium, seven volunteers were studied (Table 2). They were intubated with Ryle's tubes and their stomachs were cleaned of fasting contents. Fifty ml of saline was placed in each stomach for 14–60 min and a specimen of gastric washing obtained. Three soluble aspirin tablets (equivalent to 1 g of soluble aspirin) were then dispersed in 50 ml of saline, injected into the stomach and left for the same periods of time as saline alone. The gastric contents were then aspirated and cytological preparations made of pre- and post-aspirin specimens. These were inspected for the presence of undoubted gastric columnar cells (Croft, 1963a) which are rarely seen in gastric cytological specimens. These cells were observed much more commonly after aspirin than before (Table 2).

TABLE 2. GASTRIC EXFOLIATIVE CYTOLOGY BEFORE AND AFTER ASPIRIN

Case	Sex	Age	Diagnosis	Calcium aspirin (g)	Period of administration (min)	Gastric columnar cells	
						Pre-aspirin specimen	Post-aspirin specimen
1	M	31	Healthy volunteer	1·0	14	+	+++
2	M	54	Myocardial infarct	1·0	20	0	+
3	M	40	Hypertension	1·0	25	0	+
4	F	59	Cushing's syndrome	1·0	30	0	++
5	F	48	Depression	0·65	30	0	+++
6	F	66	Thyrotoxicosis	0·65	30	+	+++
7	M	59	Emphysema	1·0	60	0	+++

Seven volunteers were intubated. After removing fasting gastric contents 50 ml of saline was placed in stomach for 14–60 min and washings obtained for cytology. Soluble aspirin (0·65–1·0 g) was then suspended in 50 ml saline and placed in the stomach for equivalent periods and post-aspirin cytological specimens obtained. The specimens were assessed cytologically for the presence of undoubted gastric columnar cells. The frequency with which these cells were seen was indicated by +. Gastric columnar cells were found more frequently in specimens obtained after aspirin than in those obtained before it was given.

The technique was modified by washing with larger (500 ml) volumes of saline. The washings after aspirin were visibly more opaque than those before aspirin and commonly contained suspended particles (Croft, 1963a). This opacity was not due to blood and the particles were sheets of gastric surface cells and nuclei.

Four separate tests using 500 ml washings were made on a healthy medical student. In two of these no aspirin was used and, after the initial washing, fairly constant amounts of DNA were found in up to six washings. When 1 g of aspirin in 50 ml of saline was given and 5 min later the stomach was washed out and a further 1 g of aspirin given which was left in the stomach for 12 min before washing, more DNA was found in five of six subsequent washings (Fig. 1). Increased amounts of DNA were present 64 min after aspirin had been removed from the stomach. In the four tests on this student the mean DNA content of the 13 specimens obtained with no aspirin was 0·51 (s.d. 0·18) μg atoms DNA-P, and in the

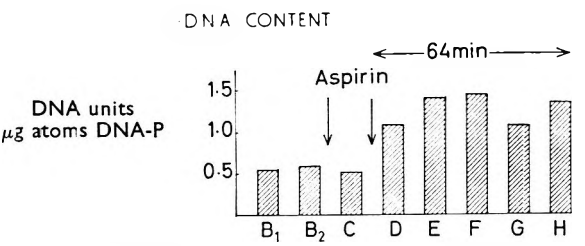

FIG. 1. A medical student was intubated with a Ryle's tube and fasting gastric contents removed with a 500 ml saline washing and discarded. 50 ml saline was placed in the stomach for 5 min and the stomach was then washed with 500 ml of saline to given specimen B_1. Following this washing 50 ml of saline was placed in the stomach for 12 min and a further 500 ml washing performed to give specimen B_2. 1 g soluble aspirin was then dispersed in 50 ml of saline placed in the stomach and left 5 min before performing a 500 ml washing (specimen C). This was followed by another 1 g dose of aspirin in 50 ml of saline which was left in for 12 min before gastric washing D. Subsequent washings E, F, G and H were obtained after placing 50 ml of saline in stomach for 5, 12, 5 and 5 min respectively. More DNA was found in five of the six washings obtained after the first dose of aspirin than was present before aspirin was administered.

nine specimens obtained after aspirin was 1·22 (s.d. 0·54) which is statistically significantly higher ($P < 0·001$).

ASPIRIN AND THE PRODUCTION OF GASTRIC EROSIONS

What effect does this excessive exfoliation have on the human stomach? Previous direct observation of the gastric mucosa, after single doses of plain aspirin tablets, by gastroscopy (Hurst & Lintott, 1939; Weiss, Pitman & Graham, 1961) and examination of the stomach after surgical excision (Muir & Cossar, 1955, 1959) indicated that the bleeding was from erosions in the mucosa. To determine the effect of repeated ingestion of soluble aspirin on human gastric mucosa, nine patients were studied at the time of elective partial gastrectomy for gastroduodenal disease (Croft & Wood, unpublished). Four patients were given three soluble aspirin tablets, dispersed in water, four times a day for 2–4 days before operation. Erosions were found in the mucosa of the resected specimens in all four patients. Erosions were found in only one of the five specimens from patients who were not given aspirin.

In the affected mucosae, one to five erosions were found, mainly along the lesser curvature and they were often joined by linear cracks which were apparent only when the specimens were stretched flat. Microscopically the erosions commonly penetrated the muscularis mucosa and blood vessels were seen in their base. When the mucosa was scraped, it stripped off readily from a specimen from a patient given aspirin, whereas the superficial layers were much tougher in the specimens from patients who had not received the drug. One patient bled post-operatively, probably from aspirin induced erosions, so it was decided not to give more patients aspirin before partial gastrectomies.

These data seemed to indicate that dispersions of soluble aspirin caused excessive exfoliation of gastric surface epithelial cells, and that if this exfoliation continued at a rate faster than the cells could be replaced an erosion would be formed.

GASTRIC SURFACE CELL TURNOVER AND OCCULT ASPIRIN BLEEDING

The relationship between cell loss and aspirin bleeding was further investigated in 15 subjects whose salicylate-induced occult bleeding had been measured by Wood (Croft, 1963b; Croft & Wood, unpublished). The technique used was based on the principles already described. Each subject was intubated with a Ryle's tube and the stomach cleaned of fasting DNA which was discarded. Fifty ml of saline was placed in the stomach which was again washed. The DNA content of these two specimens was converted to a rate by dividing the interval in minutes between each washing. Soluble aspirin (1 g) was then dispersed in 50 ml of saline and placed in the stomach for 5 min followed by another dose for 12 min, and at the end of each period a washing was made. The DNA content of these specimens was also converted to a rate of accumulation of DNA in the stomach.

Of the 15 patients, eight bled 2 ml or more per day and these were designated aspirin bleeders. Cytological preparations were not made of

specimens from one of these patients. The other seven patients bled less than 2 ml per day with repeated aspirin ingestion and these were designated aspirin non-bleeders. In one non-bleeder, only the pre-aspirin DNA rate was obtained. The results in these two groups appeared to differ in three respects (Table 3). Firstly, most bleeders appeared to have a lower

TABLE 3. THE EFFECT OF ASPIRIN ON GASTRIC SURFACE CELL EXFOLIATION IN PATIENTS SUSCEPTIBLE AND INSUSCEPTIBLE TO SALICYLATE-INDUCED OCCULT GASTRO-INTESTINAL BLEEDING

	Aspirin bleeders		Aspirin non-bleeders	
	No. patients	%	No. patients	%
Pre-aspirin gastric DNA >50 ng atoms DNA-P/min	2 (8)	25	6 (7)	86
Post-aspirin gastric DNA >100% change from pre-aspirin rate	6 (8)	75	1 (6)	17
Gastric cytology of Post-aspirin specimen Normal Abnormal	6 1 (7)	86 14	1 5 (6)	17 83

Features of gastric washings obtained before and after aspirin from 15 patients, seven of whom bled less than 2 ml/day with aspirin (non-bleeders) and eight of whom bled 2 ml or more per day (bleeders). In one non-bleeder only the pre-aspirin DNA rate was obtained and in one bleeder cytological preparations were not made. Compared with non-bleeders most bleeders had lower pre-aspirin gastric DNA rate and a greater percentage change from pre-aspirin DNA rate after aspirin. Cytologically most bleeders had normal surface epithelial cells in the post-aspirin specimen, whereas most non-bleeders had abnormal cells and inflammatory cells which indicated that they may have had atrophic gastritis. The figures in brackets are the number of patients studied for each parameter. (Abstracted from unpublished data of Croft & Wood, and also reported in Croft, 1963b.)

pre-aspirin rate of accumulation of DNA in the stomach than most non-bleeders. The mean value of 52 ng atoms DNA-P/min in the bleeders was not, however, statistically significantly different from that of 112 in the non-bleeders ($P < 0{\cdot}1$).

Secondly, most of the bleeders increased their gastric DNA after the administration of aspirin by more than 50 or 100%. Most of the non-bleeders did not; four out of six actually showed a decrease compared with the pre-aspirin rate.

Thirdly, most of the bleeders had exfoliated normal gastric surface epithelial cells in the gastric washing obtained after aspirin. Most non-bleeders had cells of a very different appearance. Numerous abnormal gastric cells and inflammatory cells were seen. These cytological changes indicated that the non-bleeders may have had atrophic gastritis.

Thus the bleeders appeared to have cytologically normal gastric mucosa with a low pre-aspirin DNA rate. After aspirin they increased their DNA rate and cytologically the specimens contained normal surface cells. The non-bleeders on the other hand mostly began with a high resting DNA rate and this tended to fall after aspirin. The high resting or pre-aspirin value suggested that there might be a high natural turnover in the mucosa of these patients. The cytological features indicated that they might have atrophic gastritis, and of the three non-bleeders who had had gastric biopsies, two of these were found to have atrophic gastritis.

Atrophic gastritis is a condition in which there is atrophy of the gastric glands and some alteration in the histology of the surface epithelium.

It occurs both in the presence and absence of pernicious anaemia; in the latter instance it is simple atrophic gastritis. In normal gastric mucosa obtained by suction biopsy from 11 patients there was a mean of 0·63 (s.d. 0·19) mitoses per 100 surface epithelial cells. Statistically significantly higher counts were obtained in atrophic gastric mucosa of patients with pernicious anaemia (1·4, s.d. 0·42, $P < 0·001$) and simple atrophic gastritis (1·5, s.d. 0·63, $P < 0·001$). These values indicated a higher turnover in atrophic than normal gastric mucosa. This was confirmed by the gastric DNA rates which were also measured in these patients and found to be higher than normal per unit surface area (Croft & others, 1966).

CONCLUSIONS

Salicylate-induced occult bleeding after repeated ingestion of aspirin appears to be the response to a physiological effect of aspirin on the gastric mucosa. It occurs from gastric erosions which result when the rate of loss of surface cells exceeds the rate at which they are replaced. A hyperdynamic mucosa, with increased rate of production or turnover of surface epithelial cells, which is probably the situation in atrophic gastritis, may confer protection against salicylate-induced occult bleeding. The mucosa in atrophic gastritis appears to be in a maximally dynamic state as a result of which aspirin cannot increase the rate of loss of cells above the mucosa's ability to replace them. However, factors other than turnover, such as alteration of epithelial cell susceptibility to aspirin, alterations in mucus production (Menguy & Masters, 1965) or achlorhydria may also be relevant to the apparent resistance of atrophic mucosa to aspirin-induced occult bleeding.

The data presented suggest that aspirin-induced occult gastrointestinal bleeding occurs because, in irritating the gastric mucosa, the drug causes excessive loss of the cells which are required to protect it. Resistance to occult aspirin bleeding may be related to gastric conditions in which there is a high natural replacement or turnover of gastric surface cells. Factors enhancing gastric mucosal susceptibility to aspirin and thus leading to severe bleeding are unknown, but may be related to gastric surface cell turnover.

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Discussion

Dr. B. B. Newbould. What proportion of severe gastrointestinal side-effects, such as ulcers, can be attributed to local concentrations of anti-inflammatory analgesic drugs, and what proportion to systemic levels? In view of the fact that many analgesic anti-inflammatory agents can cause damage to the gastrointestinal tract of animals following parenteral administration, can different methods of presentation really reduce the incidence of side-effects in man or can the lower incidence of side-effects following, for example, the administration of enteric coated tablets, be attributed to more erratic absorption? Have any observations been made on the gastrointestinal irritant effects of aspirin in patients taking cytotoxic drugs? If Dr. Croft's hypothesis on cellular proliferation is correct then aspirin should have severe effects on the gastrointestinal tract of patients receiving therapy with cytotoxic drugs.

Dr. A. J. Hale. Mitotic counts of cellular turnover rate can be extremely misleading. For example, an apparent increased cell turnover rate can be caused by arresting cells in mitosis; quite a number of agents

will cause this, so a high number of mitosing cells among non-mitosing cells may in fact refer to arrest. If there is a larger amount of DNA in the gastric washings, and the amount is not greater than twofold, this would support the idea that there was an arrest of cells in mitosis because a cell in mitosis has twice the amount of DNA than has the normal non-dividing cell, so that it would be expected that the DNA content would go up. Therefore, to argue that there is increased cell turnover from an increased mitotic count and from a limited increased amount of DNA is a dangerous thing to do.

Dr. D. G. Davey. I think Dr. Croft's paper should possibly have been called "The Action of Anti-inflammatory Substances on the Gastric Mucosa". Of the four substances he has been concerned with, probably only paracetamol is purely analgesic in action. Aspirin has analgesic and anti-inflammatory properties, phenylbutazone is similar, but I doubt if indomethacin has any analgesic action, although it is certainly anti-inflammatory. Of the four substances, then, it appears that only the pure analgesic, that is, paracetamol, was without action on the gastric mucosa.

Dr. Prescott in his talk mentioned a paper by Brodie and his colleagues (1965), concerned with the effects of, amongst other substances, indomethacin, aspirin, and phenylbutazone on the protein-binding of corticosteroids, and all appear to cause a release of corticosteroids. It is also known, as indicated by Dr. Prescott, that the corticosteroids parallel the other anti-inflammatory agents in their effect on the gastric mucosa. Would it not seem to Dr. Croft that the action of these substances on the alimentary canal, and possibly the particular action described by him, is correlated in some way with their anti-inflammatory action, and is not a simple irritant effect.

Dr. D. N. Croft. To put this in perspective, Hollander's remarks in 1946 should be stated, namely that exfoliation is a physiological response to mild irritants, and there are many substances and physical phenomena which will produce this natural physiological response of the gastric mucosa. As far as the local aspirin-like irritant effects of drugs is concerned, apart from the references I have already given, I know of no conclusive data about the proportion of patients taking drugs who develop clinical side-effects. The present formulations of soluble aspirin dissolve very rapidly, and the local effect of the macroscopic particles is not in my opinion of primary importance. The comparison of occult bleeding with major bleeding has probably led to confusion as there are other factors involved in the pathogenesis of severe bleeding. These need to be investigated. There is evidence that cytotoxic drugs reduce the mitosis rate in gut epithelium, and this may lead to an histological abnormality. It would be interesting to see if these drugs affect aspirin-induced bleeding. High mitosis counts do not prove a high turnover, and this is why we measured the rate of loss of cells in addition to the mitosis counts. In the steady state the rate of loss of cells must be in equilibrium with their production, and our data indicated that there was a higher rate of loss of cells per unit area in patients with atrophic gastritis

compared to normal. We think that the data on mitosis counts and gastric DNA rate taken together point to a high turnover in this condition. There is a 2½-fold increase in the amount of gastric DNA within 5 min of giving aspirin. This is too short a time for aspirin to have an effect on mitosis of the cell, and I think it is an irritant phenomenon.

Dr. M. J. S. Langman. Dr. Croft's evidence seems to suggest that atrophic gastritis may protect against occult bleeding. We have evidence that when erosions occur that are associated with severe bleeding they almost always appear in atrophic mucosa. We have examined gastric mucosal biopsies from people who had had aspirin bleeding and others bleeding without previous aspirin intake, and we have seen no convincing difference in the overall picture, although the erosions, in general, appeared in the atrophic mucosa. Although it seems that occult bleeding is protected against in atrophic mucosa, I am not certain that this is true in overt bleeding: indeed, the reverse may be the case.

Dr. D. N. Croft. Release of cortisone has been considered for some time as one of the methods for this response of the stomach to anti-inflammatory substances. I do not think this is the main factor. It may well be that patients with atrophic gastritis may be more prone to severe or overt bleeding.

Mr. A. W. Lessin. Is there any evidence of mucosal cells taking up aspirin, and is this possibly the reason why they come off in greater numbers? Have you looked for aspirin within the mucosal cells?

Dr. B. K. Martin. Exfoliation of cells takes place in the stomach and in the urinary tubules. I believe there is a common feature here. The kidney re-capitulates in miniature some of the processes taking place in the stomach, for re-absorption of drug occurs in the renal tubules. In both tissues the absorption of drug takes place from an acidic environment into a more or less neutral environment. Elsewhere I have advanced theoretical considerations* suggesting that the absorption of an acidic drug, from an acidic environment into a neutral environment, may well give rise to an accumulation of drug anions in the mucosal cells. It should be emphasised, however, that this will relate only to the first layer of cells. Both the gastric mucosal cells and the renal tubular cells are therefore involved in a process of drug absorption, and both may attain relatively high drug concentrations.

Dr. D. N. Croft. We have not measured aspirin in the cells, although this has been suggested by Professor Milne. It is perhaps at these two sites, stomach and kidney, that aspirin is most concentrated, the stomach because the drug remains there for a variable period during absorption and the kidney because it is being concentrated there in the process of excretion. Perhaps it is this factor of local concentration which explains why these are the sites where effects are produced.

Brodie, B. B., (1965). *Proc. R. Soc. Med.*, **58**, 946–955.

* Martin, B. K. (1963). *Nature, Lond.*, **198**, 896–897.

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The toxicity of some of the newer narcotic analgesics

R. E. LISTER

THE toxic manifestations of the newer narcotic analgesics are in many ways similar to those of the older established agents used for the relief of pain but there are some well established quantitative and qualitative differences. A full description of the pharmacology, clinical usage and side-effect liability of the narcotic analgesics was published for the World Health Organisation between 1954 and 1957 by Braendon, Eddy, Halbach & Wolff (1954, 1955, 1956, 1957) and this review will deal mainly with drugs which have been brought on to the market or introduced for clinical trial since 1957; some recent information of the toxic effects of the older established drugs will also be included. A number of critical reviews dealing with the pharmacology and clinical applications have appeared since the publication of the WHO review (Reynolds & Randall, 1957; Murphree, 1962; Martin, 1963; Foldes, Sverdlow & Siker, 1964; Lasagna, 1964; de Stevens, 1965) and these have been used extensively in the preparation of this paper. When dealing with narcotic drugs the question of toxicity is difficult to define because of the broad spectrum of effects of many of these drugs. What may be considered an undesirable or toxic effect under one set of circumstances, e.g. constipation, may be the desired effect under different circumstances. There are also additional well marked species differences in the responses to this class of drugs and extrapolation of toxic effects from experimental animals to man cannot always be justified.

In 1957 when the review by Eddy, Halbach & Braendon (1957) was published, the pharmacology, toxicology and clinical indications of most naturally-occurring, semi-synthetic or synthetic analgesics was qualitatively similar to morphine, the only exceptions to this generalisation being the narcotic antagonists nalorphine and levallorphan. Despite vast efforts by the pharmaceutical industry throughout the world no significant separation of the clinically desirable properties from the undesirable toxic effects had been achieved. All the effective analgesic drugs possessed some degree of addiction liability and in doses within the therapeutic range frequently produced unwanted effects on the central and peripheral nervous systems and adversely affected the cardiovascular, respiratory, gastrointestinal and other systems. The generalised occurrence of these effects in all the effective analgesics led many workers to believe that these effects could not be dissociated, but recent work, which will be cited in detail later, has shown that this concept is no longer tenable. This work has shown that physical dependence capacity, which is a measure of abuse or addiction liability, can be divorced from analgesic activity. Hence the term "narcotic analgesic" may be something of a misnomer, but it is retained in this context to define analgesic agents which produce

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pain relief primarily by an action on the central nervous system without producing loss of consciousness. The exact mechanism of action of this class of drugs has not yet been elucidated but the recent review by Martin (1963) gives an up-to-date account of present theories. Analgesics may be classified according to their chemical or pharmacological properties but for a logical approach a combination of the two appears to be most appropriate for dealing with the newer developments of the last few years. New drugs which have a pharmacological and toxicological profile similar to morphine will be covered first, then the group of derivatives having a chemical affinity with nalorphine will be discussed, and finally the toxicity of a miscellaneous group of drugs all of which have been found to produce pain relief in man will be mentioned briefly.

A. Morphinomimetic drugs

As their name implies these derivatives have pharmacological properties qualitatively similar to those of morphine (I) itself. When given in therapeutic doses many of these drugs produce mild toxic effects which become progressively more severe as the dose is increased. The symptoms resulting from overdosage are well characterised; respiratory depression can be demonstrated in doses only slightly larger than those required to produce pain relief (Eckenhoff & Oech, 1960) and nausea and vomiting are common; with increasing doses circulatory collapse supervenes. Central nervous system (CNS) effects are frequent and result from both excitation and depression; they include dizziness, vertigo, sedation and restlessness. Skin reactions, such as urticaria and pruritis, and sneezing are common in the less potent analgesics and appear to result from the liberation from the skin of histamine and other naturally occurring substances. True allergic reactions are uncommon in patients but occur frequently in workers who come into daily contact with the drugs. Epigastric pain, acute urinary retention and biliary colic have all been reported in these cases.

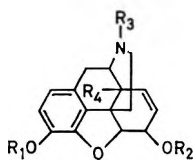
All the narcotic analgesics produce miosis in man which is thought to be the result of stimulation of the pupilloconstrictor centre or depression of an inhibitor centre. Weinstock and her colleagues (Weinstock, Stewart & Butterworth, 1958) have demonstrated that narcotic analgesics with a wide range of potencies can produce a clouding of the lens in mice and rats but this has not been reported in man. Similarly large repeated doses of narcotic analgesics are capable of producing a marked degree of corneal opacity in a variety of experimental animals (Lister, 1963, unpublished) which is reversible, but this condition has not been reported in man. Fuller details of the toxic effects of the morphinomimetic drugs will be found in a number of monographs, e.g. Reynolds & Randall (1957); Foldes, Sverdlow & Siker (1964); de Stevens (1965).

The best known and best-documented toxic effect of the narcotic analgesics is their ability to produce a condition of drug dependence commonly known as addiction. A detailed description of this toxic phenomenon is outside the scope of this review but it has been the subject of a number of recent reviews (Seevers & Deneau, 1963; Halbach & Eddy, 1963).

The degree of addiction liability of each new drug described will be indicated where it exists. Up-to-date accounts of the addiction liability of new drugs are to be found in the Minutes of the Annual Meeting of the Committee on Drug Addiction and Narcotics of the National Academy of Sciences—National Research Council.

1. DERIVATIVES OF PHENANTHRENE

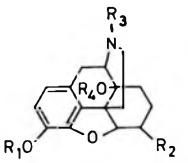
This group embraces the naturally occurring analgesics morphine (I) and codeine (II), a large group of semisynthetic derivatives ranging in potency from derivatives with an analgesic potency less than one hundredth that of morphine to the recently described derivatives of thebaine (Bentley & Hardy, 1963; Lister, 1964) some of which have analgesic potencies approaching 10,000 times that of morphine, and completely synthetic drugs of the morphinan series.



I. Morphine. $R_1, R_2, R_4 = H$; $R_3 = Me$.
II. Codeine. $R_2, R_4 = H$; $R_1, R_3 = Me$.
III. Normorphine. $R_1, R_2, R_3, R_4 = H$.
IV. Nicomorphine. $R_1, R_2 = CO$–  ; $R_3 = Me$; $R_4 = H$.
V. 14-Hydroxymorphine. $R_1, R_2 = H$; $R_3 = Me$; $R_4 = OH$.
VI. Nalorphine. $R_1, R_2, R_4 = H$; $R_3 =$ allyl.

(a) *Oxymorphone* (dihydrohydroxymorphinone). This compound (VII) and its related codeinone, oxycodone (VIII), are the only examples of analgesics, derived by substitution in the morphine nucleus, to be widely introduced into clinical practice during the last decade; they are included in the review of Eddy & others (1957) but much more work has been reported since then and the drugs have been introduced on to the world market. Oxymorphone synthesised by Weiss (1955) was shown by various workers (Coblenz & Bierman, 1956; Eddy & Lee, 1959; De Kornfeld, 1961; Keats & Telford, 1960) to be a powerful analgesic with a potency approximately ten times that of morphine. Tullar (1961) found that oxymorphone had an acute LD50 in mice 1·6 times that of morphine and suggested that it might offer a wider safety margin than morphine. Unfortunately the clinical results do not support this hypothesis. No evidence can be found to support this claim for man and there is much evidence to the contrary. Oxymorphone has been shown to be a powerful respiratory depressant in man (Resnick, Berkowitz, Rodman & Close, 1960; Lasagna, 1964) and may produce more respiratory depression than equianalgesic dose of morphine (Eddy & Lee, 1959; De Kornfeld, 1961). Keats & Telford (1960) gave reputed equianalgesic doses of morphine (10 mg/10 kg) and oxymorphone (1 mg/10 kg) to patients before operation,

and found that oxymorphone produced a statistically higher incidence of nausea and vomiting than morphine and that the incidence of other undesirable responses was also higher in the oxymorphone treated group. Oxymorphone produced marked euphoria in many patients (De Kornfeld, 1961) and possesses a high physical dependence capacity (Fraser & Isbell, 1955).



VII. Oxymorphone. $R_1, R_4 = H$; $R_2 = O$; $R_3 = Me$.
VIII. Oxycodone. $R_1, R_3 = Me$; $R_2 = O$; $R_4 = H$.
IX. Naloxone. $R_1, R_4 = H$; $R_2 = O$; $R_3 =$ allyl.
X. Hydromorphinol. $R_1, R_4 = H$; $R_2 =$  ; $R_3 = Me$.

(b) *Oxycodone* (dihydrohydroxycodeinone). This compound (VIII) bears the same relationship to oxymorphone as codeine does to morphine; it has been known for many years and Falk (1917) first reported on its clinical use. It has a potency and duration of action similar to that of morphine and its addiction liability and toxic effects are similar. A combination of oxycodone with a pectinate base (Proladone) has been introduced on to the British market as a long acting analgesic but there is no evidence that this combination has in any way reduced the toxicity of the oxycodone.

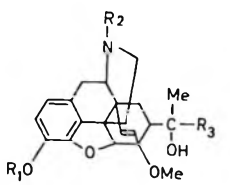
(c) *Other 14-hydroxy derivatives of morphine.* Weiss & Daum (1965) reported the synthesis of 14-hydroxymorphine (V) and hydromorphinol (X). The first was similar in potency and duration of action to morphine; the reduced derivative was about twice as potent as morphine with a prolonged duration of action in man. However no details of their toxicity or side-effect liability are yet available. Esterification of the 14-hydroxy group of oxycodone has been shown to produce an increase in analgesic potency in mice with a marked reduction in acute toxicity when compared with the parent compound (Buckett, Farquharson & Haining, 1964), but no clinical data are yet available.

(d) *Normorphine* (III). This is derived from morphine by *N*-demethylation and has been demonstrated to occur in the liver and brain of rats following the injection of morphine (Misra, Mule & Woods, 1961; Milthers, 1962) and it has been suggested as the active metabolite of morphine (Beckett, Casy & Harper, 1956). Clinical evaluation in man has shown that it is a weak analgesic possessing a potency rather less than one third that of morphine (Fraser, Wikler, Van Horn, Eisenman & Isbell, 1958). These authors also found that repeated doses of normorphine led to accumulation of toxic effects the most noticeable of which was excessive sedation. Normorphine substitutes adequately for morphine in addicts but the withdrawal symptoms are mild and it is

judged to have low physical dependence capacity (Eddy, 1959). As it offers no obvious advantages over other well established drugs it has not been used extensively.

(e) *Esters of morphine.* The nicotinic acid bis-ester of morphine (IV; nicomorphine) has been shown to possess a longer duration of action than morphine in animals and to be free of spasmogenic effects on the gut (Zirm & Pongratz, 1960). These authors have also claimed that this drug does not induce tolerance to the analgesic effects (Zirm & Pongratz, 1959). It has been suggested that it may be free from addiction liability but studies in man suggest that this is not the case.

(f) *Derivatives of thebaine.* In 1963, Bentley & Hardy (1963) showed that thebaine, an alkaloid occurring in opium but with no analgesic properties, could be used as the starting point for a series of highly potent analgesics and analgesic antagonists. These 6,14-*endo*-ethenotetrahydro-oripavine derivatives can be looked upon as bridged ring derivatives of morphine. Despite a very marked increase in potency over morphine and a marked reduction in toxicity relative to their analgesic potency in laboratory animals (Lister, 1964), the members of this series which have been tested in man show no significant qualitative advantages over morphine and in equi-analgesic doses produce probably more respiratory depression (Campbell, Lister & McNicol, 1964). These authors showed that with the derivative tested (XII; M183) there was some reduction in



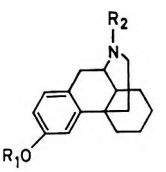
XI. M 99. $R_1 = H$; $R_2 = Me$; $R_3 = Pr$.
XII. M 183. $R_1 = COMe$; $R_2 = Me$; $R_3 = Pr$.
XIII. M 285. $R_1 = H$; $R_2 = CH_2CH\langle^{CH_2}_{CH_2}$; $R_3 = Me$.

nausea and vomiting in human volunteers and a complete absence of any histamine release, but these clinical advantages are outweighed by the undesirable respiratory distress produced. Blane (personal communication) has recently shown that M99 (XI), which is believed to be the active metabolite of M183, produced a higher neonatal mortality in rats than did morphine or pethidine when these drugs were injected into pregnant rats at term. Measurement of the oxygen consumption of the caesarean-delivered neonates indicated that respiratory depression was not necessarily the cause of death, and the author suggests that death may be associated with maternal cardiovascular disturbances during the intrauterine existence. M99 and morphine both produced a marked reduction in neonatal oxygen consumption but the neonates from the pethidine-treated mothers

showed a higher survival rate and less depression of respiration. The results suggest that in neonates there may be differences in the permeability of the brain to different drugs. Support for this view is obtained from a recent study by Way, Costley & Way (1965) who demonstrated a similar difference between the respiratory depression produced in infants after the injection of morphine and pethidine. These findings suggest that the new-born may show both quantitative and qualitative differences in their sensitivity to the toxic effects of narcotic analgesics and that this factor should be born in mind when evaluating new drugs of this type.

2. MORPHINANS

Levorphanol (XIV) was synthesised in an attempt to find a synthetic morphine-like drug devoid of some of the undesirable toxic effects of morphine, but although it had a potency about ten times that of morphine it showed no qualitative difference from morphine. Many analogues of levorphanol have been synthesised, frequently with an improvement in potency over the parent compound, but these improvements have not been matched by any significant reduction in the relative toxicity. One such drug, (−)-3-hydroxy-*N*-phenacylmorphinan methanesulphate (XV) (NIH 7525, Ro 4-0288/1) was shown to be 20 to 25 times as active as morphine in laboratory animals but only about four to five times as active in relieving post-operative pain in patients. In equi-analgesic doses no significant difference in the incidence of side-effects was found between NIH 7525 and morphine (De Kornfeld, 1960) but there is some evidence that the physical dependence capacity of this drug may be lower than that of morphine (Eddy, 1959).



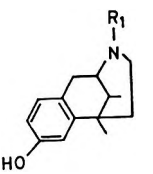
XIV. Levorphanol. $R_1 = H$; $R_2 = Me$.
XV. NIH 7525. $R_1 = H$; $R_2 = CH_2COC_6H_5$.
XVI. Levallorphan. $R_1 = H$; $R_2 =$ allyl.
XVII. Cyclorphan. $R_1 = H$; $R_2 = CH_2CH\langle^{CH_2}_{CH_2}$.

3. BENZOMORPHANS

This series of synthetic analgesics represents a further simplification of the morphine molecule. Derivatives based on this nucleus have aroused wide interest because they first provided evidence for a possible dissociation of analgesic activity and addiction liability (Eddy, 1959). Both morphine-like drugs and morphine antagonists have been developed in this series.

(a) *Phenazocine.* The initial studies on this drug (XVIII) gave rise to a great deal of optimism because, although a powerful morphine-like

drug, it was only a poor substitute for morphine in monkeys (Tedeschi, Tedeschi & Fellows, 1960) and possessed a marked separation of analgesia from cardiovascular and respiratory depression (Shemano, Wendel & Ross, 1961). However the promising results in animals have not been borne out in man. Phenazocine has been shown to be a potent respiratory depressant and when given in equi-analgesic doses it depresses respiration more than morphine or oxymorphone (Berkowitz, Rodman & Close, 1961). In man, phenazocine has been shown to substitute for morphine in addicts. Direct addiction can be induced but the physical dependence liability is less than morphine although still significant (Eddy, 1959). Sadove & Balagot (1961) reported that when used as an adjunct to anaesthesia, phenazocine produced less cardiovascular disturbance than morphine but respiratory depression and facial pruritus were observed in patients given the drug. The position of phenazocine in clinical practice has not yet been fully established. Some workers claim that it possesses a low degree of clinical toxicity (Sadove, Schiffrin & Heller, 1959; Sadove & Balagot, 1961) but others take the opposite view (Berkowitz, Rodman & Close, 1961; York, Campbell & Gordon, 1962).



XVIII. Phenazocine. $R_1 = CH_2CH_2C_6H_5$.

XIX. Pentazocine. $R_1 = CH_2\ CH = C(Me)(Me)$.

XX. Cyclazocine. $R_1 = CH_2CH(CH_2)CH_2$.

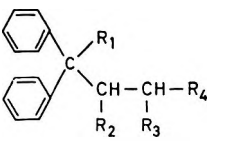
4. DIPHENYLMETHANE SERIES

Initial hopes that the synthetic analgesic methadone (amidone) (XXI) might not produce addiction soon proved unfounded but this drug has served as a model for a wide range of derivatives, a few of which have reached the stage of clinical trial and marketing.

(a) *Noracymethadol* (XXII). This congener of methadone is an effective analgesic with a more favourable oral to parenteral dose ratio than morphine and most other analgesics. Gruber & Baptisti (1963) showed that noracymethadol produced salivation, ataxia and nalorphine-reversible respiratory depression but they claimed that for an equal degree of pain relief noracymethadol produced less nausea, dizziness and drowsiness than morphine. Noracymethadol is capable of supporting physical dependence in monkeys and is thus liable to abuse.

(b) *Dextromoramide* (XXIII). Janssen & Jageneau (1957) reported on the pharmacology of dextromoramide, a new and potent analogue of methadone. Studies in the rat showed that tolerance to the analgesic

effect of dextromoramide developed slowly and that cross tolerance to morphine did not develop. As a result of this work and their own clinical observations, Soupalt and his colleagues (Soupalt, Caroli, Renon, Schops & Charbonnier, 1957) and Alvarez-Ude (1958) suggested that this drug might not prove addicting. Subsequent studies (La Barre, 1959) indicated that dextromoramide possessed an addictive potential at least equivalent to morphine. Dextromoramide is a powerful respiratory depressant (Cahal, 1958; Keats, Telford & Kurosu, 1960) and some patients appear to be unusually sensitive to this effect, many developing apnoea with analgesic doses (Black, 1966). A fatality has been reported (La Barre, 1959) from a total dose of 45 mg of dextromoramide; death was thought to be due to respiratory and cardiovascular collapse.



XXI. Methadone. R_1 = COEt; R_2 = H; R_3 = Me; R_4 = NMe_2.
XXII. Noracymethadol. R_1 = CH·O·COMe·Et; R_2 = H; R_3 = Me; R_4 = NHMe.
XXIII. Dextromoramide. R_1 = CON⟨ ⟩; R_2 = Me; R_3 = H; R_4 = N⟨ ⟩O.
XXIV. Dextropropoxyphene. R_1 = COEt; R_2 = Me; R_3 = H; R_4 = NMe_2.

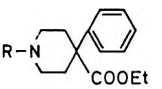
(c) *Dextropropoxyphene* (XXIV). This is a much less potent congener of methadone with an analgesic activity similar to that of codeine. Initial hopes that this compound would not be capable of supporting addiction (Gruber, 1957) have not been realised, and cases of primary physical dependence have been reported (Elson & Domino, 1963) although the incidence of drug abuse appears to be low relative to the number of doses prescribed. McCarthy & Keenan (1964) recently reported a fatality due to an overdose of propoxyphene, death being due to cardiovascular and respiratory failure accompanied by convulsions. At post-mortem generalised oedema and marked brain necrosis were found.

This drug is a mild analgesic which is effective by oral or parenteral routes but its overall toxicity and addiction liability appear to be no different from codeine (Van Bergen, North & Karp, 1960).

4. PETHIDINE DERIVATIVES

During the early 1950's organic chemists in a number of countries devoted much time and energy attempting to synthesise a safer and more potent analogue of pethidine (XXV). A number of derivatives have been made and tested, and at least four introduced clinically, but despite an increase in potency over pethidine the major toxic effects of respiratory and cardiovascular depression and emesis have invariably increased in direct proportion to the increase in analgesic activity.

(a) *Anileridine* (*Leritane*) (XXVI). This is an *N*-substituted derivative of pethidine with an analgesic potency approaching that of morphine. Pharmacological studies in dogs indicated that this drug was not emetic (Orahovats, Lehman & Chapin, 1957) but in man it was found to produce a significantly higher incidence of nausea and vomiting than equi-analgesic doses of pethidine (Chang, Safar & Lasagna, 1958). Other workers showed that it was a potent respiratory depressant and that patients found it a more unpleasant drug than pethidine, possibly because of the higher incidence of nervousness, restlessness and stimulation that followed its administration (Keats, Telford & Kurosu, 1957). Anileridine is effective by mouth but otherwise its toxic and pharmacological effects are similar to those of pethidine with an abuse liability of the same order.



XXV. Pethidine. R = Me.

XXVI. Anileridine. R = $CH_2CH_2C_6H_4NH_2$.

XXVII. Etoxeridine. R = $CH_2CH_2OCH_2CH_2OH$.

XXVIII. Piminodine. R = $CH_2CH_2CH_2NHC_6H_5$.

XXIX. Benzethidine. R = $CH_2CH_2OCH_2C_6H_5$.

XXX. Furethidine. R = $CH_2CH_2OCH_2C_4H_3O$.

XXXI. Phenoperidine. R = $CH_2CH_2CHOHC_6H_5$.

(b) *Etoxeridine* (XXVII). The pharmacology and toxicology or etoxeridine were described by Merlevede & Levis (1958), and it was found to be a potent analgesic some two to 40 times as potent as pethidine depending on the route and species used.

Initial uncontrolled clinical studies (Merlevede, 1958) indicated a low degree of clinical toxicity but in a more closely controlled study, Crawford & Foldes (1959) showed that this drug was a powerful respiratory depressant in man, producing respiratory arrest in almost every patient at a dose of 0·25–0·30 mg/kg, which was equivalent in analgesic potency to the relatively safe dose of 1 mg/kg of pethidine; cardiovascular depression was also noted by these authors. Merlevede (1958) reported three cases of drug dependence and seven cases of tolerance to etoxeridine out of 64 patients treated for carcinomatoses.

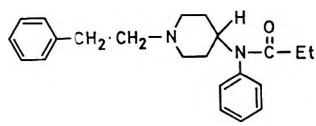
(c) *Piminodine* (XXVIII). This is a further *N*-substituted derivative of pethidine with an analgesic potency rather greater than that of morphine, but in other respects its pharmacology and toxicology resemble that of pethidine. It has a high physical dependence capacity (Woods, Deneau, Bennet, Domino & Seevers, 1961) but there is some evidence that the respiratory depression may be less than that of morphine (De Ciutiis, 1961; Lasagna, 1964).

(d) *Benzethidine* (XXIX) *and furethidine* (XXX). These are two more pethidine derivatives showing some definite advantages over pethidine when tested in animals (Lister, 1960). Both derivatives had only one

hundredth of the potency of pethidine as histamine liberators and in view of the suggestion that histamine liberation was responsible for many of the undesirable effects of pethidine (Gershon & Shaw, 1958) these compounds were tried in man. Neither drug showed any marked clinical advantage over pethidine (Masson, personal communication) and clinical trials were discontinued.

(e) *Phenoperidine* (XXXI). Rollason & Sutherland (1963) reported on the clinical pharmacology of phenoperidine. This is a potent analgesic in man but because of the unpredictability of action and incidence of side-effects in the therapeutic dose range these authors did not recommend its use. Total doses of 4 mg produced long lasting respiratory depression in some patients; others lost consciousness, although respiration appeared adequate and during this stage marked athetoid movements occurred. Catatonia with lead pipe rigidity was also reported. Vertical nystagmus was reported in 66% of the patients and there was a high incidence (17%) of nausea and vomiting and respiratory depression (20%). This drug has also been used in neuroleptanalgesia (Ingvar & Nilsson, 1961) but this combination has now been replaced by newer and safer preparations.

(f) *Fentanyl* (R 4263) (XXXII). Fentanyl can be looked upon as a pethidine analogue; it is a highly potent, short acting analgesic with an activity some 200 times that of morphine (Janssen, 1962; Gardocki & Yelnosky, 1964). In mice the therapeutic index of 775 compares very favourably with that for morphine of 31 but insufficient evidence is available to show if this favourable ratio applies to man. Because of its high potency and short duration of action it has been used in combination with droperidol for neuroleptanalgesia under the trade name of Innovar (Holderness, Chase & Dripps, 1963). The advantages and disadvantages of neuroleptanalgesia with drug combinations of this type have recently been discussed in a symposium devoted to this topic (Shepherd, 1965).



XXXII. Fentanyl.

Apnoea requiring ventilation is commonly observed with this treatment and muscular rigidity and laryngospasm, making ventilation difficult, has also been reported (Foldes & others, 1964). The technique of neuroleptanalgesia is still experimental and further work is required before a full evaluation of the hazards associated with it can be fully appraised.

B. Narcotic antagonists

Substitution of the methyl group on the tertiary nitrogen atom of many analgesics by unsaturated three carbon moieties, e.g. allyl, frequently

gives a competitive antagonist to the original analgesic. Much interest has been aroused recently by the findings that some of the antagonists are analgesics in their own right with pharmacological spectra which differ in many respects from that of the original parent compound. These antagonists are usually devoid of addiction liability and if taken by a narcotic-dependent subject precipitate an intense withdrawal syndrome.

1. DERIVATIVES OF PHENANTHRENE

(a) *Nalorphine* (VI). Early studies on nalorphine indicated that this drug was an effective antagonist to the narcotic actions of morphine (Hart, 1941; Unna, 1943). It was capable of reversing the analgesia, respiratory depression and miosis produced by morphine, and found a ready use as an antagonist to the narcotic analgesics in cases of overdosage. Nalorphine has been shown to be free of addiction liability (Isbell, 1956) and Lasagna & Beecher (1954) showed that it was a powerful analgesic with a potency similar to that of morphine. However, because of the high incidence of toxic side-effects which develop in many patients following an effective analgesic dose, it has not proved to be a practical proposition as an analgesic. When nalorphine is given alone it frequently produces marked psychotomimetic effects (Lasagna & Beecher, 1954; Woods, 1956; Keats & Telford, 1956) which have been attributed to a CNS stimulant effect. The psychotomimetic effects described include anxiety, dysphoria and visual hallucinations with occasional panic due to a sense of impending death. Morphine has little effect on these psychic disturbances but they can be abolished by pentobarbitone or chlorpromazine. Nalorphine shows other stimulant actions on the CNS; it can cause emesis, myosis and bradycardia and give rise to convulsions in experimental animals (Woods, 1956). Excessive doses of nalorphine given after morphine have induced convulsions in a female patient (Wolfe, 1955). Nalorphine alone is a powerful respiratory depressant (Foldes & others, 1964) and although effective in antagonising the respiratory depression produced by morphine overdosage, it may, if the existing depression is severe, fail to antagonise and even potentiate the depression (Campbell, 1965, personal communication). Nalorphine can produce hypotension which may be severe in some patients (Eckenhoff, Elder & King, 1952).

(b) *Naloxone* (IX). This is a newly introduced derivative of oxymorphone. It is a powerful narcotic antagonist approximately 30 times as potent as nalorphine as an antagonist (Foldes, Lunn, Moore & Brown, 1963) and shows some degree of group specificity against oxymorphone (Sadove, Balagot, Hatano & Jobgen, 1963). Naloxone has recently been shown (Lasagna, 1965) to possess some analgesic properties, 2 mg naloxone giving pain relief similar to that observed with 10 mg morphine. Strangely, higher doses produced less effect and 8 mg produced less pain relief than expected from a placebo. There have been no reports of psychotomimetic effects with this drug and it appears to be free from addiction liability (Foldes & Torda, 1965).

(c) *M*285 (XIII). This is one of a series of potent and long lasting analgesic antagonists derived from thebaine and related to M99 (XI) described by Bentley, Boura, Fitzgerald, Hardy, McCoubrey, Aikman & Lister (1965). It is a weak analgesic of the nalorphine type but possesses powerful and long lasting psychotomimetic properties (Eddy, 1965 and Campbell & Lister, 1965, unpublished observations).

(d) *Levallorphan* (XVI). Levallorphan is a more specific narcotic antagonist than nalorphine and even in large doses has no analgesic or psychotomimetic effects. Although it may produce mild respiratory depression (Foldes & Torda, 1965), no other toxic effects have been reported. It is free of addiction liability but is capable of precipitating the withdrawal syndrome in narcotic addicts. Levallorphan has been combined with narcotic analgesics, especially pethidine, with the object of antagonising the respiratory depression of the narcotic without reducing the analgesia to the same extent. Studies in volunteers (Herxheimer & Sanger, 1957) and patients (Foldes, McNall, Koukal & Tanaka, 1959) indicated that this might be possible, but recent controlled studies have shown that it is unlikely (Telford & Keats, 1961; Campbell, Masson & Norris, 1965). These latter authors showed that in some circumstances levallorphan may potentiate the respiratory depression due to pethidine. Differences in the duration of action of the analgesic and the antagonist may lead to a dangerous state of renarcotisation in a patient previously thought to be free from respiratory depression.

(e) *Cyclorphan* (XVII). This morphinan derivative is a powerful analgesic with similar properties to cyclazocine, but may prove to be even more potent as an analgesic (Deneau & Seevers, 1964; Lasagna, 1965). Like cyclazocine and pentazocine, cyclorphan can produce psychic disturbances.

(f) *Pentazocine* (XIX). Substitution of the tertiary nitrogen in the benzomorphan nucleus by allyl and other unsaturated groups gave a series of narcotic antagonists (Archer, Albertson, Harris, Pierson, Bird, Keats, Telford & Papadopoulos, 1962; Harris & Pierson, 1956, 1964). Pentazocine was found to be a weak narcotic antagonist with significant analgesic properties when tested against post-operative pain in man (Telford, Papadopoulos & Keats, 1961; Keats & Telford, 1964; Stoetling, 1965). These authors found that 10–20 mg/70 kg of pentazocine produced analgesia equivalent to that of 10 mg/70 kg of morphine. The incidence of subjective side-effects was qualitatively and quantitatively similar to those produced by morphine; only one of 75 patients described any nalorphine-like effects and these were minimal. Pentazocine produced significantly less nausea than morphine and the incidence of vomiting was also low. On the other hand pentazocine produced a high incidence of hypertension and tachycardia, conditions not observed after morphine although these conditions could not necessarily be attributed to the drug treatment (Sadove, Balagot & Pecora, 1964). Both morphine and pentazocine induced respiratory depression although that produced by pentazocine could not be antagonised by nalorphine. Restlessness, pruritis and signs of histamine release were observed in patients given

pentazocine but the incidence appeared to be no greater than with morphine.

Fraser & Rosenberg (1964) demonstrated that the addiction liability of pentazocine was low, approximating to that of dextropropoxyphene. It would not substitute for morphine in addicts and, when given by chronic administration to post-addicts, only one subject elected to continue taking the drug when offered the chance to discontinue it, even though he showed a severe inflammatory reaction at the injection site. When the drug was stopped this subject showed a mild abstinence syndrome. Thus pentazocine has been shown to be the first clinically potent analgesic with minimal psychotoxicity and addiction liability, and gives hope for the future development of a safe potent analgesic which may be free of addiction liability and the other distressing side-effects so long associated with the narcotic analgesics.

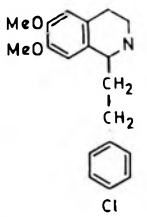
(g) *Cyclazocine* (Win. 20,740) (XX). This compound is a potent narcotic antagonist with powerful anticonvulsant, sedative and muscle relaxant properties (Harris & Pierson, 1964; Weiss & Laties, 1964). It has been found to be a highly effective analgesic when given either orally or parentally in doses as low as 0·25 mg, which can produce pain relief equal to 10 mg morphine (Lasagna, De Kornfeld & Pearson, 1964), but it is capable of producing mental confusion, depersonalisation and dysphoria reminiscent of nalorphine. Although it can give rise to respiratory depression the slope of the dose response curve is not parallel to, and is shallower than that found for morphine, and the ceiling effect appears to be much lower; thus the possibility of dangerous respiratory depression due to overdosage with this drug appears to be less than with the older analgesics. These authors also pointed out that this drug, like pentazocine, had minimal addiction liability. An abstinence syndrome has been precipitated in experimental subjects given repeated doses of cyclazocine (Lasagna, 1965). These subjects who were all post-addicts found that the initial and subsequent subjective effects of this drug were so unpleasant that there would appear to be little chance of abuse of this agent by potential or established addicts.

C. Miscellaneous analgesics

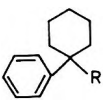
This group comprises a heterogenous collection of drugs which have been reported to produce pain relief in man but which have no direct chemical affinity with the established narcotic analgesics. However, as they all seem to act on central nervous structures they have been included.

(a) *Methopholine* (Versidyne, Ro4-1778/1) (XXXIII). This drug is an isoquinoline derivative chemically related to the analgesically inactive opium alkaloid papaverine. Methopholine has an analgesic activity similar to that of codeine (Sadove, Schiffrin & Ali, 1961). The addiction liability of this drug is very low and probably less than that of codeine (Fraser, Martin, Wolbach & Isbell, 1961). In man, methopholine produces respiratory depression which is accompanied by a mild degree of cardiovascular depression. There is evidence that the respiratory depression produced by this drug is not antagonised by levallorphan but instead the

two drugs produce an additive depression of respiration (Foldes, Moore & Suna, 1961). These workers also reported a severe atopic allergic reaction in a patient, which was relieved by hydrocortisone. This drug produces pain at the injection site and is usually given orally and often in combination with aspirin, but a high percentage (30%) of patients reported nausea with this mixed dose regimen (Cass & Frederik, 1964).



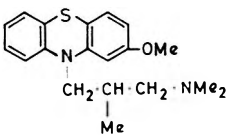
XXXIII. Methopholine.



XXXIV. Phencyclidine.

R = N

XXXV. Cl 400.
R = NHEt.



XXXVI. Methotrimeprazine.

(b) *Phencyclidine* (XXXIV) and *Cl* 400 (XXXV). These drugs are closely related members of a series of cyclohexylamines which are capable of producing a high degree of sensory blockade at the sub-cortical level. Profound analgesia sufficient for minor surgical procedures can be induced with doses of 0·25 mg/kg body weight (Johnstone, Evans & Baigel, 1959; Collins, Gorcspe & Rovenstine, 1960). Unlike the morphinomimetic drugs the cyclohexylamines do not give rise to respiratory or cardiovascular depression and may even stimulate these functions, nor do they substitute for morphine in addicts.

Despite these apparent advantages, the high incidence of toxic effects associated with the administration of these drugs has precluded their widespread clinical use. These effects were mainly the result of stimulation of the CNS which took the form of marked agitation and hallucinosis and in many cases a state of catatonic stupor developed. Hypertension and tachycardia frequently accompanied the hallucinatory state and appeared to be secondary to it as termination of the hallucinations with barbiturates abolished both effects. The hallucinations were usually unpleasant and often accompanied by nausea and salivation and the effects showed a marked similarity to those seen in chronic schizophrenics (Cohen, Rosenbaum, Luby & Gottlieb, 1962). The incidence of psychotomimetic effects may approach 50% in adult patients treated with these drugs but there is a lower incidence of undesirable effects in the elderly. Phencyclidine provided adequate analgesia in 78% of infants being treated for burns (Muir, Evans & Mulcahy, 1961) but these authors reported a 50% incidence of hallucinations and crying in the children.

This drug has been withdrawn from clinical use but it has demonstrated that profound analgesia can be produced without necessarily incurring the risks of addiction and respiratory depression.

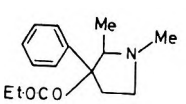
(c) *Methotrimeprazine* (XXXVI). Methotrimeprazine is a phenothiazine derivative which has been found to be an analgesic with a potency when given parentally similar to that of morphine (Lasagna & De Kornfeld, 1961; Motilla, 1963). It appears to be devoid of addiction liability (Fraser & Rosenberg, 1963) and respiratory depression (Pearson & De Kornfeld, 1963). It is ineffective when given orally but when given parenterally it may give rise to pain at the site of injection; marked sedation and giddiness have been reported in volunteers (Pearson & De Kornfeld, 1963). As this drug is a phenothiazine, its toxicity is likely to resemble that of this group of drugs rather than the narcotics and a careful watch must be kept for toxic effects such as blood dyscrasias and hepatic involvement. Hollister (1965) recently reviewed the toxicity of this group of drugs and stresses the need for care in prescribing these potent agents.

(d) *Prodilidine* (Cogesic) (XXXVII). This drug can be regarded as having some chemical affinity with pethidine but it is a much weaker analgesic. Cass & Frederik (1963) found prodilidine to be only one half as potent as codeine on a weight basis.

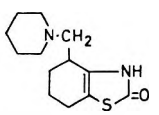
Doses of 50 mg prodilidine produced pain relief in 69% of ambulatory patients studied by Batterman, Mowratoff & Kaufmann (1964), but when the dose was increased to 75 mg the increased incidence of undesirable side effects led to a reduced patient acceptance and pain relief was found in only 61% of patients.

The physical dependence capacity of prodilidine is low, reflecting its weak analgesic properties, and no clinical evidence of abuse has been reported to date.

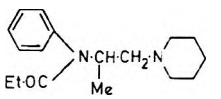
(e) *SU*4432 (XXXVIII). This compound was found to be an analgesic of similar potency to codeine when tested in man against post-operative pain. Clinical trials were terminated because a small percentage of patients complained of blurred vision believed to be due to inflammation of the optic nerve (de Stevens, 1965). Normal vision returned on cessation of the drug treatment.



XXXVII. Prodilidine.



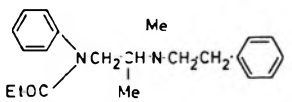
XXXVIII. SU 4432.



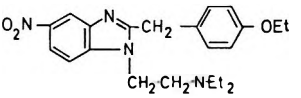
XXXIX. Phenampromide.

(f) 2-*Amino-indane* (*SU* 8629) (XLII). This simple indane derivative was found to have an analgesic potency about 20% that of morphine when tested in animals (Witkin, Heubner, Galdi, O'Keefe, Spitaletta & Plummer, 1961). These authors found the drug to have certain similarities to amphetamine, it was not antagonised by nalorphine and further studies indicated that it would not substitute for morphine in morphine-tolerant monkeys. Although found to be effective as an analgesic when given orally in man it produced an undesirable incidence of central stimulation and hypotension which led to the abandonment of further clinical studies (de Stevens, 1965).

(g) *Benzimidazoles.* These form a group of highly potent analgesics, of which an example is etonitazine (XLI). Although these compounds are effective analgesics in man they produce marked respiratory depression in analgesic doses and have a high addiction potential. These disadvantages were sufficient to warrant termination of clinical trials (de Stevens, 1965).



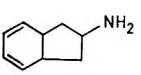
XL. Diampromide.



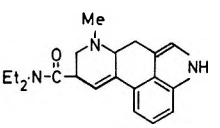
XLI. Etonitazine.

(h) *Phenampromide group.* A series of basic anilides were reported to have analgesic action by Wright, Brabander & Hardy (1959). Of this series two derivatives, phenampromide (XXXIX) and diampromide (XL) were tested clinically and found to have analgesic activity similar to pethidine and morphine respectively. Both compounds possessed morphine-like properties but possessed no obvious clinical advantages over morphine and have not been marketed.

(i) *Lysergic acid diethylamide* (LSD 25) (XLIII). This drug is a powerful psychotomimetic agent producing euphoria in some subjects. Kast & Collins (1964) tested it as an analgesic agent in patients with severe pain and found that 0·1 mg LSD 25 produced significantly greater pain relief in the third hour after administration than either 2 mg dihydromorphinone or 100 mg pethidine.



XLII. SU 8629.



XLIII. Lysergic acid diethylamide.

Three patients out of 50 reported nausea and vomiting with LSD 25; psychotic reactions occurred in most of the patients but these were not judged to be sufficiently severe to warrant their termination with chlorpromazine. Despite prolonged pain relief eight patients refused a second dose of the drug because of the unpleasant psychic effects. There was no evidence of cardiovascular or respiratory depression with this drug and it does not support morphine dependence. There is, however, increasing evidence that this drug is liable to abuse, but evidence of true drug dependence is lacking.

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Discussion

Mr. A. W. Lessin. Dr. Lister, you talk about separation of analgesic properties and addiction liability, but on looking at the examples you have given, the addiction liability seems to be related to the potency in all cases except pentazocine, and I wonder whether the confidence with which you stated that there was a separation might not be shaken.

Dr. H. O. J. Collier. I think it is agreed that, if the rate of onset of analgesia is the same for two drugs, potency in inducing dependence about equals analgetic potency. Where potency is high, the social danger of a drug is high, because its illicit exchange is easier. Heroin is thus more dangerous socially than morphine, and a compound a thousand times more potent would be very much more socially dangerous. This raises an important question. Should we seek analgetics of high potency, if these are liable to be equally highly addictive? Wisely or luckily, the very potent analgetics recently discovered have not so far been introduced into medicine.

Dr. Cicely Saunders. Much of the toxicity of this group of drugs depends not only on their chemistry but also on the way in which the drug treatment is managed. The toxic features of this group of drugs include dependence, and we may take diamorphine as one which at least has the reputation of being more likely than any other drug to cause dependence. Yet of the last 48 patients who were with us for longer than three months, only nine ever needed more than 10 mg doses; for 20 patients the 10 mg dose was the maximum, while 19 received less than 10 mg dosage. So that over many weeks this drug has been used without producing this type of toxicity. Whatever new drug is introduced its management forms a major part of the treatment. So many trials have been single dose studies, and as Lasagna and others have pointed out, dependence is usually assessed on addicts or post-addicts and other side-effects on normal volunteers. Just as it is difficult to transfer toxicity effects from animals to man, it is also very difficult to transfer observations made on addicts or volunteers, to patients with chronic pain. There is a need for trials which are patient-centred rather than drug-centred, in which the long-term effects and side-effects are assessed.

Dr. R. E. Lister. I did point out that the physical dependence capacity of pentazocine was virtually zero, similarly with cyclazocine, and also a drug which I have examined, M285. These three compounds are narcotic antagonists of different potencies, and none of them support morphine-type physical dependence: in fact in every instance they would precipitate withdrawal symptoms in addicts who tried to use them. Therefore these are examples in which dissociation of analgesia and physical dependence is possible. There is no evidence that some of the other compounds, for example methotrimeprazine and phencyclidine and drugs like the two SU compounds produce physical dependence. But there is increasing evidence that it is possible to dissociate the clinical analgesia from physical dependence capacity. I agree with Dr. Collier.

The assumption that diamorphine, for example, is a more potent drug of addiction than say morphine has been questioned by Isbell and some of his colleagues, who claimed that if the drugs were given in equi-active doses there was no evidence that diamorphine was a stronger drug of addiction than morphine in addicts and post-addicts.

I think the assessment of the addiction potential and dependence capacity in patients raises an ethical question, and it has been suggested that patients are perhaps not the best subjects for this kind of study because the actual incidence of narcotic addiction in patients is low compared with the rate of dependence in so-called normal people, and there may well be a relation between dependence and the actual physical process of introducing a euphoric agent into the body.

Chairman's Summary

There is a strong impression that these drugs can produce toxic effects, and I think the onus is on the people who are using them, or those who prescribe them for their patients, to be aware of this and perhaps to act on the assumption that toxic effects can be produced until it has been clearly shown not to be so.

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Partial molal volumes of some non-ionic detergents in monomeric and micellar form

A. T. FLORENCE

Partial molal volumes of five synthetic non-ionic detergents have been measured above and below their critical micellar concentrations. The increases in volume on micellisation are discussed in terms of changes in hydration and hydrophobic bonding. The change in partial molal volume at the critical micellar concentration decreased with temperature. At 20° increases of 5, 9 and 17 ml $mole^{-1}$ were obtained for the n-butyl, n-hexyl and n-octyl hexaoxyethylene glycol ethers respectively. For comparison with the alkyl ethers, the partial molal volume of hexaoxyethylene glycol was determined at four temperatures. The utility of density measurements for the determination of the critical micelle concentration of these non-ionic detergents depended on the temperature of the system; at higher temperatures the change in slope of density-concentration plots at the critical micelle concentration decreases and sometimes is not detectable.

THE partial molal volumes of ionic surface-active agents increase on micelle formation (Wright & Tartar, 1939; Paquette, Lingafelter & Tartar, 1943; Harkins, Mattoon & Corrin, 1946; Shinoda & Soda, 1963) to the extent of 10 to 22 ml $mole^{-1}$ of detergent. No similar data have been obtained for polyoxyethylene glycol alkyl ethers and this communication sets out to provide information on the partial molal volumes of some members of this class of non-ionic detergents above and below their critical micellar concentrations (CMC). This information is important because changes in partial molal volume in aqueous solution must be related to changes in the state of the monomer, particularly to changes in hydrophobe-water interactions (Némethy & Scheraga, 1962).

Density measurements have been used to determine the CMC of ionic detergents (Harkins & others, 1946). How far this method is useful for obtaining the CMC of non-ionic surfactants has been assessed.

Experimental

The compounds examined have been described previously (Elworthy & Florence, 1964, 1965). The straight chain detergents have a general formula $Me{\cdot}[CH_2]_x{\cdot}[OCH_2CH_2]_6{\cdot}OH$ with x = 3, 5 and 7 (designated C_4n_6, C_6n_6 etc.) and the two branched chain detergents $R_2{\cdot}CH{\cdot}CH_2{\cdot}[OCH_2{\cdot}CH_2]_6{\cdot}OH$ with R = Me or Et designated Me_2n_6 and Et_2n_6. Cetomacrogol 1000 (Evans Medical) was recrystallised from an acetone-ether mixture. Hexaoxyethylene glycol (n_6) was prepared as before and purified by repeated fractional distillation.

A Lipkin pycnometer of approximately 11 ml capacity was used to measure the density of the solutions, according to the procedure of Bauer & Lewin (1960). A smaller pycnometer (0·5 ml capacity) was used for pure liquids. Each density was the mean of at least two determinations. The precision obtained on repeat measurements at 20° was $\pm 0{\cdot}00003$ g ml^{-1} with a temperature control of $\pm 0{\cdot}01°$.

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Water for calibration of the pycnometers and preparation of the solutions was once distilled from potassium permanganate solution.

Results

Density measurements on solutions of Me_2n_6 were made over the whole range of concentrations and the results are recorded in Fig. 1. The deviation from linearity at the CMC can clearly be seen. Measurements on the other systems were confined to a maximum of 8% w/w concentration.

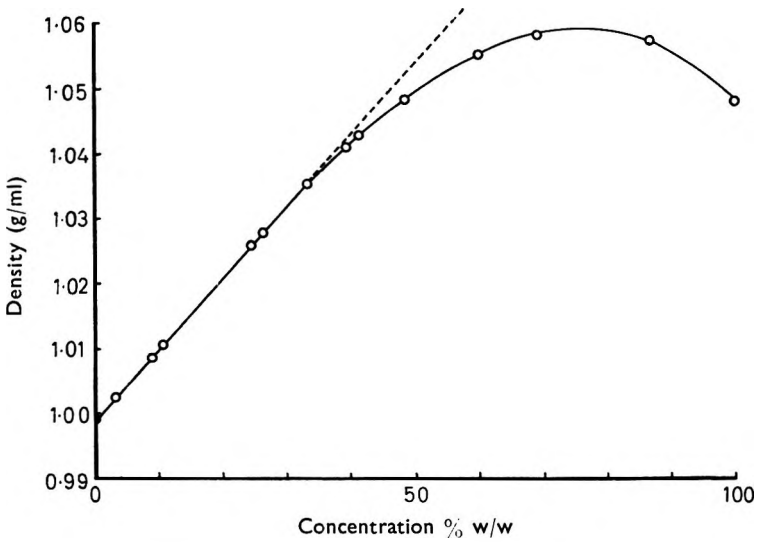

FIG. 1. The variation of the density of solutions of Me_2n_6 at 20° with concentration. The deviation from linearity begins at the CMC (34% w/w) and the density passes through a maximum at 74% w/w, the latter probably being due to a change in structure of the solution.

Density-concentration plots are given in Fig. 2 for C_6n_6 and C_8n_6. The effect of temperature on the density plots of the two detergents is shown in this figure. The change of slope in the plots at the CMC of C_8n_6 is significant at 20°, indicating a CMC of 0·38% w/v compared with a value of 0·42% w/v extrapolated from the results of Corkill, Goodman & Ottewill (1961); at 30° the break is much less pronounced and at 40° is hardly detectable using the present technique. Bury & Parry (1935) found the change of slope in density plots for potassium laurate at the CMC to be smaller at 35° than at 25° and forecast that at higher temperatures the change would become too small to be detected. Lal (1953) confirmed this trend with lauryl sulphonic acid.

The changes in slope of the density-concentration plots are reflected in the changes in the partial molal volumes (PMV) of the detergents at the CMC. Calculated values of PMV of pure liquid detergent, monomeric detergent and micellar detergent are listed in Table 1.

It will be seen that there is a decrease in molal volume as the liquid detergent or glycol is placed in solution. Such decreases on solution have been ascribed to the high internal pressure of water (Masterton, 1954). On micellisation the alkyl chains of the monomers are removed from their aqueous environment and they expand causing increases in partial molal volume. These are recorded in the last column of Table 1.

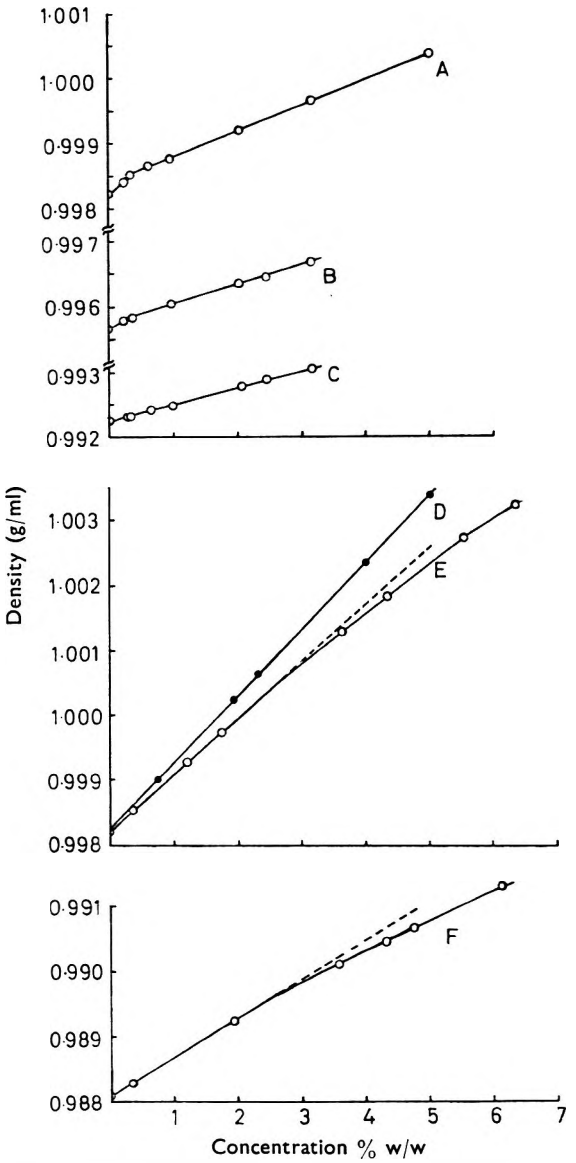

FIG. 2. Density-concentration plots of: A, C_8n_6 at 20°; B, C_8n_6 at 30°; C, C_8n_6 at 40°; D, cetomacrogol 1000 at 20°; E, C_6n_6 at 20°; F, C_6n_6 at 50°C.

TABLE 1. PARTIAL MOLAL VOLUMES OF PURE LIQUID, MONOMERIC AND MICELLAR DETERGENTS (ml mole^{-1})

Compound	Temperature °C	V_0 pure liquid state (molal volume)	$\bar{V}$ monomeric state	$\bar{V}m$ micellar state	Change on micellisation ml mole^{-1} $\bar{V}m-\bar{V}$
Me_3n_6	20°	323	302	308*	6
Et_2n_6	20°	360	336	341	5
C_4n_6	20°	324	302	307*	5
	30°	327	305	—	–
	40°	331	—	—	–
C_6n_6	20°	362	333	342	9
	30°	364·7	341	345	4
	40°	367·3	345	347	2
	50°	369·6	347·3	352·7	5·4
C_8n_6	20°	397·2	363	380	17
	30°	399·2	377	383	6
	40°	400·9	384	387	3
Cetomacrogol 1000	20°	—	†	1085	–
	30°	—	†	1099	–
Hexagol	20°	250·6	238	—	–
	30°	252·1	244	—	–
	40°	253·6	246	—	–
	50°	255·0	247	—	–

* Measured in the region up to 6% w/w above the CMC as PMV of these two compounds increases slowly with concentration.

† The CMC of cetomacrogol 1000 is too low to measure the PMV below this concentration. The results on micellar solutions are included to verify the trend of volume increases with short chain-length compounds.

The increase in volume is greater the longer the alkyl chain and in general becomes smaller with increase in temperature.

The partial molal volumes of the monomers ($\bar{V}$), which do not vary with concentration within the limits of experimental error, are much smaller than those of the pure liquids (V_0) and $\bar{V}-V_0$ becomes more negative, as might be expected, as the alkyl chain length increases. The contraction of hexagol on solution at 20° amounts to 12·6 ml mole^{-1}. Breuer (1964) has estimated that the formation of a hydrogen bond in aqueous solution is accompanied by a contraction of 1·05 ml mole^{-1}. This would suggest that, in the present instance, there were twelve hydrogen bonds involved. As six water molecules have been found to hydrate the hexaoxyethylene glycol chain (Elworthy & Florence, unpublished results), an arrangement of the water molecules involving these twelve hydrogen bonds may be envisaged. While, at this stage, it is not possible to be unequivocal about the arrangement of the hydrating water and the hydrogen bonds, as there is the possibility of some intramolecular bonding involving the terminal hydroxyl group, we may relate $\bar{V}-V_0$ to hydration. On this basis, $\bar{V}-V_0$ for this glycol at 30° (−8·1 ml mole^{-1}) corresponds to 4 water molecules of hydration, a figure which compares favourably with the viscosity estimate of 4·5 molecules.

The contribution of the glycol chain in each of the synthetic detergents may be assumed to be identical and the remainder of the contraction in solution may be assumed to be due to the hydrocarbon chain. A mechanism for the volume reduction of non-polar solutes in aqueous solution has been given by Némethy & Scheraga (1962), who computed that the formation of a hydrophobic bond—a partial reversal of the solution process—was accompanied by a volume increase ΔV_H which is a function of the number of water molecules ΔY hydrating the hydrocarbon chain.

At 20°, $\Delta V_H = 0{\cdot}78\ \Delta Y$ ml mole^{-1}. Using this equation it is possible to find ΔY knowing ΔV_H from Table 1. The values of ΔY so found should be of use in attempts to quantify the thermodynamics of the micellisation process.

Discussion

Except for the C_4n_6 and Me_2n_6* compounds the partial molal volumes of the compounds examined were constant in micellar solutions. Very slight increases were evident with Et_2n_6 and C_6n_6. According to Shinoda & Soda (1963) constancy of the PMV above the CMC coincides with a pseudophase separation model of micelle formation. Activity measurements on C_4n_6, Me_2n_6 and Et_2n_6 indicate that the pseudophase model does not hold for these compounds (Elworthy & Florence, unpublished results). The present behaviour agrees with this view.

It might be expected that the volume of the monomer would increase on micellisation by the same amount as the contraction attributed to the alkyl chain on solution (Table 2) because micellisation is a reversal

TABLE 2. ESTIMATES OF THE NUMBER OF WATER MOLECULES HYDRATING THE ALKYL CHAINS OF THE DETERGENT MONOMERS (20°)

Detergent	Contraction on solution due to alkyl chain, ml	Number of water molecules ($\Delta V_H/0{\cdot}78$)
Me_2n_6	9	12
Et_2n_6	11·5	15
C_4n_6	10	13
C_6n_6	17	23
C_8n_6	22	29

of the solution process as far as the alkyl chain is concerned. But the changes are much less. For example, with C_6n_6, the increase in volume on micellisation is only 9 ml mole^{-1} whereas the decrease in volume due to the hydrocarbon chain-water interaction is 17 ml mole^{-1}. This may be the result of incomplete removal of the alkyl chains from contact with water or an increase in the solvation of the polyoxyethylene chains.

The results for the effect of temperature on the change of PMV at the CMC (Table 1) show clearly that the change on micelle formation diminishes with increasing temperature. This is to be expected as the water structure breaks down. The non-linear increase in PMV of the monomers may be explained after Masterton (1954) on the basis of two competing processes affecting the alkyl chain: (i) the normal linear expansion of the molecules and (ii) the breakdown of the cage-like water structure around the alkyl chains. The result for C_6n_6 at 50° is anomalous and at this stage it is impossible to say whether this is due to a change in micellar state or not. The trend of results shown here may be related to enthalpy and entropy changes in similar systems. Crook, Fordyce & Trebbi (1963) have observed that the entropies of micellisation of a series of *p*-t-octyl phenol polyoxyethylene ethers decrease with temperature and were all negative above 65°, implying changes in water structure.

* PMV of Me_2n_6 at 50% w/w = 313 ml mole^{-1}, at 70% = 318·5 ml mole^{-1}

As a means of determining CMC's, density measurements provide a bulk property which is a valuable alternative to surface measurements. However, difficulties are caused by the low CMC's of most non-ionic detergents and by the fact that the alteration in slope of the density plots at the CMC is subject to the temperature of the system. Often the change in slope is almost impossible to detect (e.g. C_8n_6 at 40°) rendering the method of little use, unless more precise determinations of density are made in dilute solutions by a more accurate technique such as the magnetic float method. This technique would extend the scope of density measurements to a wider range of detergents than those quoted here.

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A periarterial nerve-circular muscle preparation from the caecum of the guinea-pig

P. I. AKUBUE

A periarterial nerve-circular muscle preparation from the caecum of the guinea-pig has been described. Stimulation of the periarterial nerve produced relaxation of the circular muscle strip. These responses were blocked by adrenergic neurone blocking agents but not by ganglion blocking drugs. Phenoxybenzamine or piperoxan greatly reduced the relaxation but propranolol abolished it. No relaxation was observed on preparations from animals pre-treated with reserpine. It was observed that after the blockade of the responses to periarterial nerve by adrenergic neurone blocking drugs, stimulation of the periarterial nerves in the presence of physostigmine induced contractions which were blocked by local anaesthetics or hyoscine but not consistently by ganglion blocking drugs.

IN 1930, Finkleman described the effect of periarterial nerve stimulation on the isolated intestine of the rabbit. He recorded the responses of the longitudinal muscle and obtained relaxation and less frequently contractions. Since that time, many workers have examined the nature of the responses and also the effect of drugs on these responses (Day & Rand, 1961; Gillespie & MacKenna, 1961; Bentley, 1962; Boyd, Gillespie & MacKenna, 1962). The effect of the periarterial nerve stimulation on the circular muscle does not seem to have been examined in detail. Garry & Gillespie (1955) reported that the stimulation of the sympathetic nerve to the rabbit colon inhibited both muscle coats. Van Harn (1963) obtained contraction or relaxation of the circular muscle of the cat intestine on stimulation of the sympathetic nerve.

A periarterial nerve-circular muscle preparation of the guinea-pig caecum is described and the nature of the responses to the nerve stimulation has been investigated.

Methods

Guinea-pigs weighing about 500 g were killed by stunning and bleeding. The preparation of the perivascular nerve-caecal circular muscle strip is described in Fig. 1.

The periarterial nerve was stimulated with supramaximal voltage (about 50 V) at a frequency of 50 shocks/sec and at a pulse duration of 0·1 msec unless otherwise stated. The stimulation was for 20 sec every 4 min and this timing was automatically controlled with an 'Interval Timer' (Electrical Remote Control Co. Ltd.) The responses were magnified 10 times and were recorded on smoked paper with an isotonic frontal-writing lever.

Drugs. The drugs used were bethanidine sulphate, bretylium tosylate, dexamphetamine sulphate, dimethylphenylpiperazinium iodide, guanethidine sulphate, hexamethonium bromide, hyoscine hydrobromide, (−)-noradrenaline bitartrate, pentolinium tartrate, phenoxybenzamine

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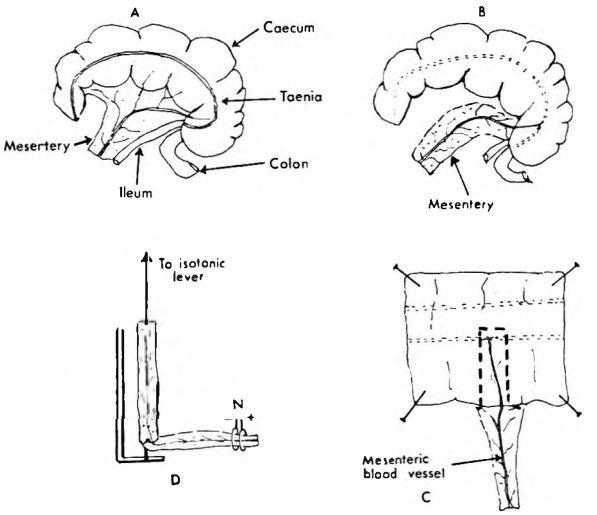

FIG. 1. The preparation of the perivascular nerve—caecal circular muscle strip of the guinea-pig. The upper drawing on the left (A) shows the caecum with parts of the ileum, the colon and the mesentery with its blood vessels. One of the taeniae is also shown. The taenia caeci distant to the mesentery were dissected and the ileum separated from the mesentery. The mesentery with its blood vessels was freed from the end of the caecum distal to the ileo-caecal junction to a point about 1 cm from the junction (B). The caecum was then cut open along its length near the mesenteric border so that the mesentery remained attached to the opened caecum. It was washed in Krebs solution and pinned out with the mucosal surface downwards on a cork pad in the Krebs solution. The drawing "C" shows a part of the caecal wall with the mesentery. One of the blood vessels running from the mesentery across the caecal wall is shown. The caecal circular strip 4 cm long by 4 mm wide was cut to include this blood vessel as shown in "C". The end of the strip near the mesentery was tied to a holder. The preparation was then suspended in an organ bath containing 80 ml Krebs solution at 37° gassed with 95% oxygen 5% carbon dioxide. The other end of the strip is attached to an isotonic lever (D). The mesentery with its blood vessels was pulled through two platinum ring electrodes (Birmingham & Wilson, 1963) for the perivascular nerve stimulation (N).

hydrochloride, physostigmine salicylate, piperoxan hydrochloride, propranolol hydrochloride and reserpine. The concentrations of all the drugs are expressed as final bath concentrations in μg/ml of the base.

Results

Stimulation of the periarterial nerve to the caecal circular muscle strip at a pulse duration of 0·1 sec produced inhibition of the preparation. The response increased with the frequency of stimulation from 12 shocks/sec and was maximal at about 50 shocks/sec. It also increased with pulse duration from 0·01 msec to reach a maximum at 0·3 msec. These responses were not obtained when the mesentery between the electrodes and the circular muscle was cut.

The action of ganglion blocking drugs. The inhibitory responses to periarterial nerve stimulation were not modified by hexamethonium or pentolinium in concentrations up to 100 μg/ml.

The effect of α- or β-*adrenergic receptor blocking agents.* Fig. 2 illustrates the typical effect of phenoxybenzamine or propranolol on the responses to perivascular nerve stimulation. Phenoxybenzamine (0·1 μg/ml) greatly reduced the responses but a higher concentration (5 μg/ml) did not abolish them. The relaxation was reduced by propranolol (5 μg/ml) and eliminated by 10 μg/ml of propranolol. Piperoxan (5–10 μg/ml) reduced but did not abolish the responses.

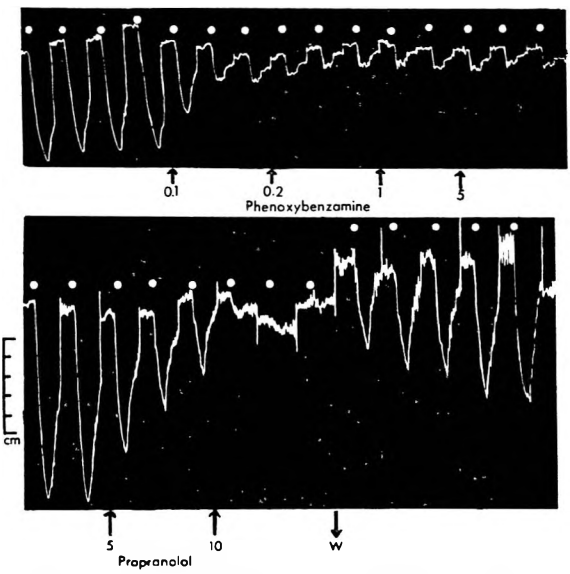

FIG. 2. The effect of phenoxybenzamine and propranolol on the responses of the caecal circular muscle to perivascular nerve stimulation. The preparations were stimulated for 20 sec every 4 min at a frequency of 50 shocks/sec, with a pulse duration of 0·1 msec and supramaximal voltage. The numbers represent the concentration of the drugs in μg/ml of the bath fluid. Phenoxybenzamine (0·1 μg/ml) greatly reduced the relaxations but increasing the concentration to 0·2, 1 or 5 μg/ml did not abolish the responses (upper tracing). Propranolol (5 μg/ml) greatly reduced the responses and a higher concentration (10 μg/ml) abolished them. When the preparation was washed, the responses partly returned.

The influence of adrenergic neurone blocking drugs. Guanethidine (2-8 μg/ml) blocked the responses to periarterial nerve stimulation. This blockade persisted after washing out the antagonist drug and was only slightly reversed by the addition of dexamphetamine (1–10 μg/ml). The inhibitory responses always returned when dexamphetamine was washed out of the bath. When dexamphetamine (2 μg/ml) was present in the bath, guanethidine (2 μg/ml) did not modify the responses of the circular muscle to periarterial nerve stimulation (Fig. 3).

Blockade of the responses of the circular muscle strips was produced with bretylium (4–8 μg/ml), bethanidine (3 μg/ml) or dimethylphenylpiperazinium (5 μg/ml). The effects were partially reversed by dexamphetamine (1–10 μg/ml) but were completely reversed when dexamphetamine was washed out.

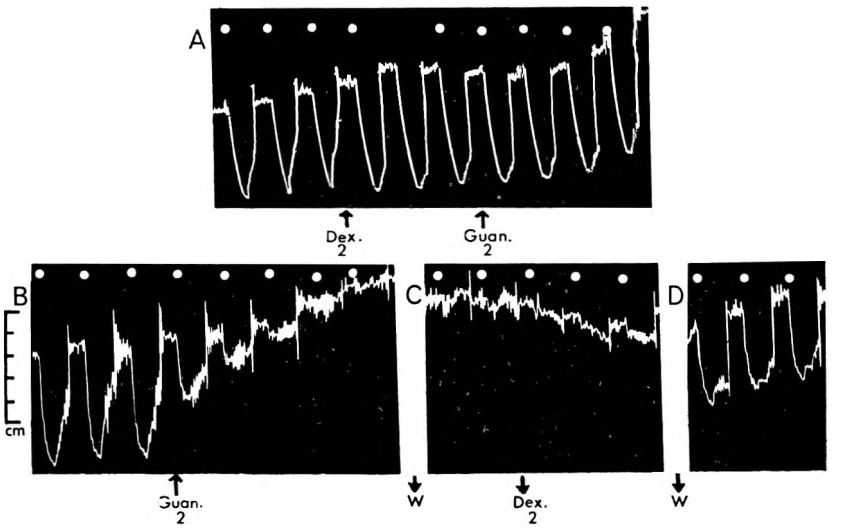

FIG. 3. The effect of guanethidine (Guan) and dexamphetamine (Dex) on the responses of the caecal circular muscle to perivascular nerve stimulation. The parameters and the intervals of stimulation were the same as in Fig. 2. The upper record shows the effect of guanethidine (2 μg/ml) on the responses of circular muscle in the presence of dexamphetamine (2 μg/ml). Dexamphetamine slightly enhanced the relaxations and guanethidine did not modify the relaxations. The lower records show the effect of guanethidine (2 μg/ml) on the responses. The relaxations were slowly abolished. The responses did not return when the preparation was washed (between B and C). The responses only slightly returned in the presence of dexamphetamine (2 μg/ml) in "C", but after washing out the dexamphetamine, complete recovery of the responses was obtained (D).

The effect of pre-treatment with reserpine. Four guinea-pigs were treated with reserpine, 5 mg/kg daily for two days by intraperitoneal injection, and killed on the third day. Little or no response to periarterial nerve stimulation was elicited in any of the preparations but noradrenaline produced inhibition of these circular muscle strips.

Contractions to periarterial nerve stimulation. It was observed that after washing out the adrenergic neurone blocking agent, stimulation of the periarterial nerve in the presence of physostigmine (0·1 μg/ml) induced contractions of the preparation. The contractions were usually small and were not increased by increasing the voltage to 100 V or reducing the frequency to 6 or 12 shocks/sec. These contractile responses were reduced by hexamethonium (100 μg/ml) in two experiments or by pentolinium (50–100 μg/ml) in two out of four experiments. Dimethylphenylpiperazinium (5 μg/ml) blocked the contractions in two experiments but only reduced them in six others. The contractions were blocked by cocaine (30 μg/ml) or hyoscine (0·1 μg/ml) or by cutting the mesentery between the electrodes and the circular muscle. The effect of pentolinium or hyoscine is shown in Fig. 4.

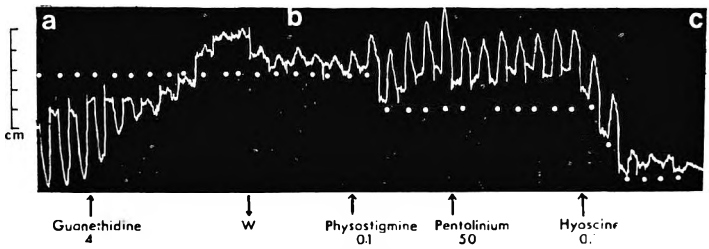

FIG. 4. The contractions of the caecal circular muscle preparation to perivascular nerve stimulation. The perivascular nerve was stimulated with a pulse duration of 0·1 msec and supramaximal voltage. Between "a" and "b" the frequency of stimulation was 5 shocks/sec and between "b" and "c" the frequency was 12 shocks/sec. The intervals of stimulation was as in Fig. 2. The numbers represent the doses of the drugs in μg/ml of the bath fluid. Guanethidine (4 μg/ml) slowly abolished the relaxations to perivascular nerve stimulation. When the preparation was washed at W, very small contractions to perivascular nerve stimulations were obtained. Note that the contractions did not increase when the frequency of stimulation was reduced to 12 shocks/sec. In the presence of physostigmine (0·1 μg/ml), the contractions were enhanced. These contractile responses were only reduced by pentolinium (50 μg/ml) but were abolished by hyoscine (0·1 μg/ml).

Discussion

Stimulation of the periarterial nerve to the caecal circular muscle produced relaxation of the preparation. These inhibitory responses were blocked by adrenergic neurone blocking drugs. Day (1962) reported that dexamphetamine prevented or reversed the effect of adrenergic neurone blocking drugs on the responses to sympathetic nerve stimulation. In the present experiments, dexamphetamine prevented the effect of these blocking drugs but reversed their effects only after it was washed out of the bath.

The inhibitory responses were almost abolished by phenoxybenzamine or piperoxan. Propranolol blocked the responses. This is indicative of catecholamine involvement in the response. However, propranolol is known to have local anaesthetic activity (Morales-Aguilera & Vaughan Williams, 1965) and this perhaps played a part in its blocking action.

Reserpine pre-treatment eliminated the relaxation to the periarterial nerve stimulation. This again provided additional evidence for the adrenergic nature of the response. Gillespie & MacKenna (1961), and Bentley (1962) reported that reserpine pre-treatment not only prevented the relaxation of the longitudinal muscle of the rabbit gut to sympathetic nerve stimulation, but also converted the responses to contractions. No contractions were observed on the circular muscle strips.

The responses of the caecal circular muscle to periarterial nerve stimulation resembled in many respects those of the longitudinal muscle. Finkleman (1930) found that the inhibition of the rabbit intestinal segments was antagonised by ephedrine. When adrenergic neurone blocking drugs became available it was shown, as in the present experiments, that the responses were blocked by these agents (Boura & Green, 1959, 1963; Maxwell, Plummer, Schneider, Povalski & Daniel, 1960; Bentley, 1962; Wilson, 1962; Birmingham & Wilson, 1965).

Garry & Gillespie (1955) stated that stimulation of the lumbar sympathetic nerve to the rabbit colon caused inhibition of the circular muscle. A similar observation has been made on the cat gut (Van Harn, 1962). The present experiments provided evidence for the adrenergic innervation of the caecal circular muscle. Probably other intestinal circular muscles are similarly innervated. Norberg (1964) found that most adrenergic nerves within the gut wall terminated in the plexuses. It is likely therefore that the transmitter released on stimulation of the periarterial nerve diffuses to the circular muscle cells to activate the receptors.

It has been found that adrenergic neurone blocking drugs converted the responses of the intestinal longitudinal muscle to sympathetic nerve stimulation from inhibition to contraction (Day & Rand, 1961; Bentley, 1962; Boyd & others, 1962). A similar observation has now been made on the circular muscle. Day & Rand (1961) found that the contractions of the rabbit ileum to periarterial nerve stimulation revealed by guanethidine were not always blocked by hexamethonium. They suggested that their results represented a cholinergic link in the sympathetic mechanism according to the hypothesis of Burn & Rand (1959). The contractions of the rabbit gut to sympathetic nerve stimulation produced by guanethidine or after pre-treatment with reserpine were shown by Bentley (1962) to be blocked by hexamethonium. Bentley favoured the suggestion of Gillespie & MacKenna (1961) that the contractions were due to stimulation of the preganglionic parasympathetic fibres. The results obtained on the circular muscle did not disprove or confirm either of the above possibilities. The inconsistency of the results with ganglion blocking drugs made the interpretation difficult. Probably the nerves were cholinergic since the responses were blocked by hyoscine.

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Effect of bradykinin on the human uterus *in vitro*

SIR,—Compounds inhibiting the motility of the human uterus are of therapeutic interest. Only a few compounds are so far known to exert this effect, for example isoxsuprine (Lish, Hillyard & Dungan, 1960; Bygdeman & Eliasson, 1963a), prostaglandin E (Bygdeman & Eliasson, 1963b) (see also Lehrer, 1965). Landesman, Campbell & Wilson (1963) reported that bradykinin in 0·2–0·4 μg/ml of bath fluid also caused a pronounced inhibition of the motility of isolated strips from non-pregnant, and also pregnant human uteri. These results were at variance with those reported by Berde & Saameli (1961), who could not record any effect of bradykinin on the human uterus *in vitro* or *in vivo*. The present study was made to further elucidate the possible effect of bradykinin on the human uterus *in vitro*.

Myometrial strips were obtained from uteri removed because of myoma. At least four pieces of apparently normal myometrium (2 × 2 × 20 mm) were taken longitudinally from the corpus of each uterus. Each strip was suspended in a 40 ml cuvette containing oxygenated modified Tyrode solution. Temperature (37·5°), pH (7·35 ± 0·05) and other parameters were kept constant (Bygdeman & Eliasson, 1963b; Bygdeman, 1964). Synthetic bradykinin was dissolved in Tyrode solution and tested on three strips from three different uteri. Bradykinin did not exert any effect on the spontaneous motility in concentrations up to 10 μg/ml bath fluid. The other strips from the same uteri responded in the usual way to prostaglandin E and other compounds tested.

Saameli and Hendricks have also told me that they could not find any effect of an intravenous infusion of 0·5 μg bradykinin/kg/min on the human uterine motility *in vivo*.

The results from the present study *in vitro* are in agreement with those reported by Berde & Saameli (1961) but at variance with those of Landesman & others (1963). The explanation for the discrepancies is not known but may lie with the choice of solvent; it is known that the usual solvent for the synthetic polypeptides made available by Sandoz has a pronounced inhibitory action on the motility of human uteri *in vitro*.

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Reversal of adrenergic vasodepression

SIR,—Nearly twenty years ago Coret & Van Dyke (1948) reported the reversal of the adrenergic vasodepression observed in the cat after blockade of what are now called α-receptors, by doses of isoprenaline, 1·0 mg/kg. To explain this phenomenon they proposed the hypothesis that vascular smooth muscle was furnished with both excitatory and inhibitory "reactive patches," and that the adrenolytic drugs probably blocked most, but *not all*, excitatory patches and no inhibitory patches. The 1·0 mg/kg dose of isoprenaline was thought to remain loosely combined with the inhibitory patches for several minutes during which time they were inaccessible, but adrenaline still had access to the excitatory patches which had escaped the blocking drug. They suggested that the loose combination of isoprenaline and inhibitory patches gradually broke down so that the latter were again free to react with adrenaline and the depressor response returned. Since Butterworth (1963) has demonstrated the β-adrenergic blocking action of isoprenaline, these results of Coret & Van Dyke would seem to be quite comparable with those of Hull, Eltherington & Horita (1960) using the β-blocking agent, dichloroisoprenaline, and with those of Moreira & Osswald (1965) using the β-blocking agent, pronethalol. Although different terminologies are used, the proposed mechanisms would appear to be similar.

The experiments reported here demonstrate the phenomenon of "tapenolysis" in the femoral vascular bed independently of cardiac or central nervous system effects, or both.

Dogs of 9 to 15 kg were anaesthetised with pentobarbitone sodium, 32·5 mg/kg. Blood flow in the femoral artery was measured with an electromagnetic flow meter (Medical Avionics Model 6000) and recordings were made on a Honeywell Visicorder (Model 1508); intra-arterial injections were made through a polyethylene tube inserted into a small branch of the femoral artery. Isoprenaline hydrochloride, (–)-adrenaline bitartrate, (–)-noradrenaline bitartrate, phenoxybenzamine hydrochloride, dichloroisoprenaline hydrochloride, and pronethalol hydrochloride were the drugs used. The doses are expressed as the salts.

After the intra-arterial injection of 0·5–1·0 mg/kg of phenoxybenzamine the vasoconstrictor effect of adrenaline was changed to a purely vasodilator effect (Fig. 1). The subsequent injection of pronethalol, 100–300 μg/kg intra-arterially, caused a re-reversal of the adrenaline effects. The vasodilator effects of isoprenaline were markedly diminished by the treatment with intra-arterial pronethalol. We have found in the femoral vasculature, as others have found in systemic blood pressure, that "tapenolysis" is, with time, a completely reversible phenomenon.

In the ten experiments described above, the vasoconstrictor response to noradrenaline was unchanged, or only slightly reduced, by the phenoxybenzamine. In four other experiments the vasoconstrictor response was totally blocked by phenoxybenzamine and "tapenolysis" could not be demonstrated, i.e. intra-arterial doses of pronethalol up to 500 μg/kg which blocked the vasodilator response to isoprenaline blocked, but did not reverse, the vasodilator response to adrenaline.

In eight additional experiments the blockade of β-receptors in the leg was maintained by an intra-arterial infusion of dichloroisoprenaline (0·02–0·05 ml/min of a 0·5% solution). Under these experimental conditions the intra-arterial administration of phenoxybenzamine blocked the vasoconstrictor responses to adrenaline and noradrenaline equally. This is to be contrasted with the normal situation where it was found that the vasoconstrictor response to adrenaline could be reversed by a dose of phenoxybenzamine which had little or no effect on the vasoconstrictor response to noradrenaline.

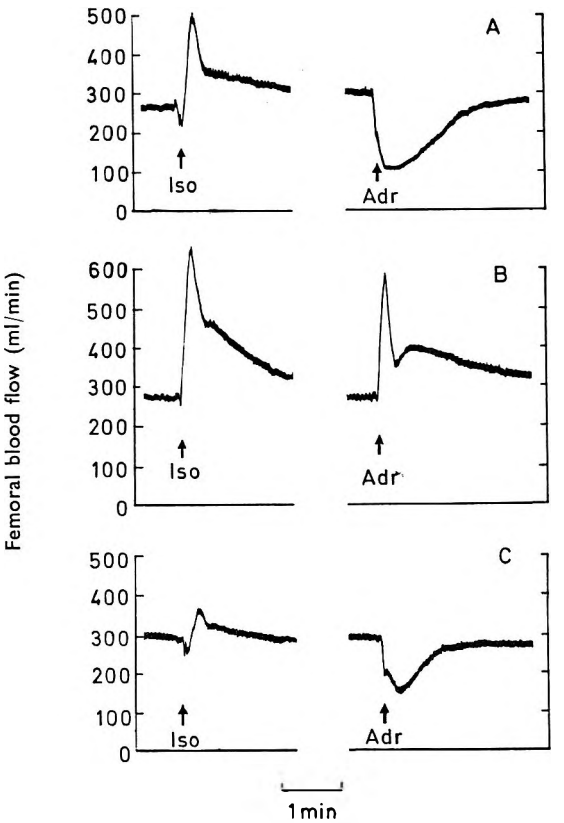

FIG. 1. Blood flow in the femoral artery of a dog, measured with an electromagnetic flowmeter. Drugs administered intra-arterially through a polyethylene tube inserted into a small arterial branch. Isoprenaline (Iso), 0·05 μg/kg i.a., administered at 09.07 hr in A, 10.05 hr in B and 10.36 hr in C. Adrenaline (Adr), 0·1 μg/kg i.a., administered at 09.12 hr in A, 10.12 hr in B and 10.23 hr in C.

Phenoxybenzamine, 500 μg/kg i.a., was administered between A and B. Pronethalol, 300 μg/kg i.a., was administered between B and C.

An attempt was made to demonstrate "tapenolysis" in the femoral vascular bed in four experiments using ephedrine instead of a β-blocking agent. Intra-arterial doses of 100–500 μg/kg of ephedrine did not affect the vasodilator response of the femoral vascular bed to adrenaline after phenoxybenzamine. Nor was the vasodilator response to isoprenaline affected by the ephedrine.

It would seem that there are two different mechanisms for the reversal of adrenergic vasodepression. The first involves the reversal of the depressor action of adrenaline in phenoxybenzamine-treated animals and is induced by β-blocking agents. It is demonstrable in the femoral vasculature. The second involves a reversal of the vasodepressor action of isoprenaline in normal animals, and is induced by a variety of vasoconstrictor substances (Lands, Luduena, Grant, Ananenko & Tainter, 1950; Walz, Koppanyi & Maengwyn-Davies, 1960; Levy & Ahlquist, 1961). An alteration of the availability of α- and β- receptors, as first proposed by Coret & Van Dyke (1948), would seem to

explain the first phenomenon; increased cardiac output in the presence of a sustained vasoconstriction, as first suggested by Lands & others (1950), would seem to explain the second phenomenon.

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Effects on lipomobilisation of the β-adrenergic blocking drugs, propranolol and INPEA

SIR,—An increase of plasma free fatty acids (FFA) occurred within 60 min after subcutaneous administration of propranolol [1-isopropylamino-3-(1-naphthyloxy)-2-propanol hydrochloride] (Black, Crowther, Shanks, Smith & Dornhorst, 1964) to rats. The rise was more evident with low doses and disappeared with increasing dosage (Table 1). In contrast, (±)-INPEA (*N*-isopropyl-*p*-nitrophenylethanolamine hydrochloride) (Somani & Lum, 1965) diminished plasma FFA at lower doses while, at greater doses, it did not induce significant changes in FFA level. The results obtained with the two optical isomers seem to indicate that a mild lipid-mobilising power is linked only to (−)-INPEA (Table 1).

The lipomobilising activity of propranolol *in vivo* was prevented by previous reserpinisation or treatment with dibenzyline (Table 2). Thus propranolol action on lipolysis *in vivo* is apparently an indirect adrenergic one.

Regarding the antagonistic action against the noradrenaline-induced lipomobilisation, propranol and (±)-INPEA are equally active *in vivo* (Table 3). The inhibitory power of INPEA appears to be greater in the (−)-isomer (Table 3).

In vitro propranolol and INPEA did not show any intrinsic lipomobilising activity on rat epididymal adipose tissue. On the contrary, at high concentrations (2 and 20 × 10^{-5}M) they depressed the basal lipolytic activity.

The antagonism of propranolol and INPEA against the FFA mobilisation stimulated by noradrenaline *in vitro* was studied according to a procedure previously described (Fassina, Tóth & Santi, 1965). The curves obtained by plotting the log concentration of noradrenaline against the amount of FFA released in the presence of increasing concentrations of propranolol and INPEA, indicate that the two β-adrenergic blocking drugs behave as competitive antagonists. The pA_2 values (Schild, 1947) (calculated when the effect of noradrenaline was 50% of the maximal) show that (−)-INPEA ($pA_2 = 6{\cdot}32$) is less active than propranolol ($pA_2 = 6{\cdot}75$) whilst (+)-INPEA ($pA_2 = 4{\cdot}20$) has a very small activity. From these values the affinity of (−)-INPEA for the lipid mobilising sites affected by noradrenaline gives results about 130 times higher than that of (−)-INPEA and 3 times lower than that of propranolol. This

striking quantitative dependence of the competitive antagonism on the steric configuration of the ethanolamine side-chain indicates that this part of the molecule of INPEA is involved in occupying the specific active sites for catecholamines in adipose tissue.

From the comparison of propranolol, (+)-INPEA and (−)-INPEA it seems that (*a*) propranolol has a greater lipomobilising action *in vivo* than INPEA, (*b*) propranolol is more active *in vitro* than INPEA, (*c*) the increase in FFA and the antagonistic action are both greater in the (−)-isomer of INPEA. These facts suggest that the lipomobilising and the antiadrenergic properties are related. The question now arises how the two actions may be connected. Other β-adrenergic

TABLE 1. EFFECT OF PROPRANOLOL AND INPEA ON PLASMA FREE FATTY ACID (FFA) LEVEL IN RATS

Drug	Dose and route	FFA % variation*	P†
Propranolol	2 mg/kg s.c.	+ 58 ± 9	<0·01
	5	+ 54 ± 8	<0·001
	40	+ 15 ± 6	n.s.
(±)-INPEA	1·65 mg/kg s.c.	− 30 ± 6	<0·01
	4·1	− 22 ± 3	<0·01
	33·0	+ 9 ± 5	n.s.
(−)-INPEA	2 mg/kg i.p.	+ 17 ± 3	<0·02
	10	+ 40 ± 6	<0·001
(+)-INPEA	2 mg/kg i.p.	− 8 ± 8	n.s.
	10	− 8 ± 3	n.s.

Male Sprague-Dawley fed rats (200 ± 30 g) were used. Animals were killed 60 min after treatment s.c. and 30 min after treatment i.p. Subcutaneous doses of propranolol and (±)-INPEA are equimolar, corresponding respectively to 7, 17 and 135 μM/kg. FFA were determined according to Dole (1956).

* Each value represents the mean ± s.e. of 5 to 12 rats.

† P = significance of the difference from the control (saline treated) group.

TABLE 2. EFFECT OF RESERPINE AND DIBENZYLINE ON THE FREE FATTY ACID (FFA) MOBILISATION INDUCED BY PROPRANOLOL IN RATS

Treatment	FFA % variation*	P†
Propranolol	+ 85 ± 4	<0·001
Reserpine + propranolol	− 18 ± 3	<0·05
Dibenzyline + propranolol	+ 1 ± 2	n.s.

Propranolol, 10 mg/kg i.p. 30 min before killing. Reserpine, 3 mg/kg i.p. repeated twice, 40 and 15 hours before propranolol. Dibenzyline, 10 mg/kg i.p. 2 hr before propranolol.

* Each value represents the mean ± s.e. of 8 rats.

† P = significance of the difference from the respective control group (treated with saline or with antagonistic drug + saline).

TABLE 3. ANTAGONISTIC EFFECT OF PROPRANOLOL AND INPEA ON THE INCREASE OF PLASMA FREE FATTY ACIDS (FFA) INDUCED BY NORADRENALINE IN RATS

Treatment	FFA % variation*	P†	Inhibition
Noradrenaline	+ 85 ± 2	<0·001	—
Propranolol + noradrenaline	+ 31 ± 2	<0·001	64%
(±)-INPEA + noradrenaline	+ 29 ± 5	<0·01	66%
Noradrenaline	+ 80 ± 7	<0·001	—
(−)-INPEA + noradrenaline	+ 36 ± 4	<0·001	55%
(+)-INPEA + noradrenaline	+140 ± 7	<0·001	0

Noradrenaline, 0·5 mg/kg i.p. 30 min before killing. Propranolol, 40 mg/kg s.c. 15 min before noradrenaline. (±)-, (+)- and (−)-INPEA, 33 mg/kg s.c. 15 min before noradrenaline. The doses of propranolol and INPEA are equimolar.

* Each value represents the mean ± s.e. of 8 rats.

† P = significance of the difference from the respective control group (treated with saline or with the antagonist + saline).

blockers (DCI and pronethalol) show a lipomobilising action together with a blocking effect against the catecholamine-induced FFA mobilisation. However, these compounds also stimulate lipolysis *in vitro*. These contrasting actions are generally ascribed to a direct dual effect on adipose tissue—a lipolytic and an antiadrenergic one—depending on the dose (Fröberg & Orö, 1963; Love, Carr & Ashmore, 1963; Westermann & Stock, 1963; Schusterová, Krčíková, Mühlbachová, Hynie & Wenke, 1964; Kvam, Riggilo & Lish, 1965; Love, Carr & Ashmore, 1965). On the other hand, propranolol is completely devoid of a direct lipolytic action *in vitro*, while its lipomobilising action *in vivo* is an indirect adrenergic one. The low content of endogenous catecholamines in adipose tissue (Paoletti, Smith, Maickel & Brodie, 1961; Sidman, Perkins & Weiner, 1962; Stock & Westermann, 1963) could explain the lack of FFA-releasing effect of propranolol *in vitro*. A similar condition exists with some indirect sympathomimetic amines, such as tyramine and amphetamine, which show a noticeable lipomobilising activity *in vivo* consequent to catecholamine release from nerve ending stores (Westermann & Stock, 1963; Fassina, 1964), but fail to manifest any lipomobilising action *in vitro*. Furthermore, they also antagonise the lipid mobilising effect of noradrenaline *in vitro* (Mühlbachová, Wenke, Schusterová, Krčíková & Elisová, 1964). The last fact seems to indicate that these drugs have some affinity for the receptor sites for catecholamines in adipose tissue (Mühlbachová & others, 1964). This affinity, being low, is completely masked *in vivo* by the catecholamine releasing action. From this comparison I am led to consider that propranolol behaves in a similar way to indirect acting sympathomimetic amines, but that it differs in intensity of lipomobilising effect *in vivo* and antiadrenergic action *in vitro*, probably because of a greater distribution coefficient and affinity towards the adrenergic receptors in adipose tissue than to the catecholamine stores. This difference appears to be further emphasized in (−)-INPEA.

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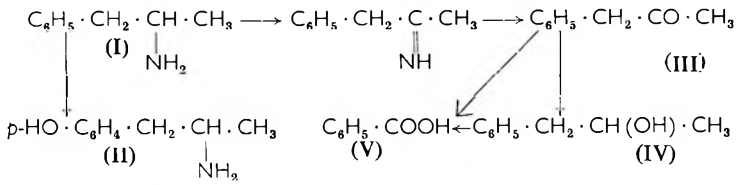
The fate of amphetamine in man and other mammals

SIR,—Axelrod (1954a,b; 1955) showed that amphetamine (I) could be metabolised along two routes; by aromatic hydroxylation to *p*-hydroxyamphetamine (II) and by deamination to benzyl methyl ketone (III). This ketone could then yield 1-phenylpropan-2-ol (IV) *in vivo*, and it has been shown in this laboratory (Smith, Smithies & Williams, 1954; El Masry, Smith & Williams, 1956) that it is, in fact, metabolised to (+)-1-phenylpropan-2-ol and benzoic acid, whilst the alcohol is partly oxidised to benzoic acid (V) and partly conjugated with glucuronic acid. The main pathways of amphetamine metabolism (excluding conjugation) could be expressed in this scheme.



p-Hydroxyamphetamine and benzoic acid have been shown to be urinary metabolites of amphetamine (Axelrod, 1954b; Alleva, 1963) but benzyl methyl ketone and alcohol have not. If one allows for deamination and hydroxylation occurring in the same molecule and for stepwise oxidation of the side-chain, then theoretically, amphetamine could give rise *in vivo* to 25 or more metabolites.

We are examining the urinary metabolites of ^{14}C-amphetamine (α-methyl-[β-^{14}C]-phenethylamine) in detail in man, the rat, rabbit and greyhound, and our preliminary results are reported here. ^{14}C-labelled (+)-, (−)- or (±)-amphetamine sulphate in aqueous solution was administered orally or by intraperitoneal injection. ^{14}C excretion was estimated by end-window and scintillation counting and the excretion of metabolites by paper chromatography and reverse isotope dilution.

Table 1 shows that most of the ^{14}C is eliminated in the urine mainly in the first 24 hr after dosing, and after 3 days nearly all the ^{14}C has been excreted.

In the rat, the figures in Table 1 suggest that the ^{14}C from the (−)-isomer may be excreted a little slower than from the other isomers, but the difference does not appear to be significant.

Table 2 shows that aromatic hydroxylation is the major metabolic reaction of amphetamine in the rat whereas deamination is the major reaction in man, rabbit and dog. Amphetamine itself is a major excretory product in man. The urinary outputs of the drug in the three subjects examined were 23, 33 and 35% of the dose, or an average of about 45% of the ^{14}C excreted in 24 hr. Unchanged amphetamine is also a major excretory product in the one dog examined, the amount being 38% of the dose or about 43% of the 24-hr excretion of ^{14}C. In the rabbit, the excretion of unchanged amphetamine is low (4% of the dose in 24 hr), but in the rat there is a moderate excretion of about 15% of the dose in 48 hr. It has been shown by others (Asatoor, Galman, Johnson & Milne, 1965; Beckett, Rowland & Turner, 1965) that the amount of amphetamine excreted unchanged by man and the rat depends on the pH of the urine, more being excreted in an acid than an alkaline urine. In our experiments, the human urines had pH values of 6·2–6·8. The urines from the rats, rabbits and the dog were collected during 24 hr after dosing, and after this time the pH was about 7·5 for the rats and the dog and about 8 for the rabbits. Freshly collected urines from rabbits had values of about pH 6, which rose on standing at room temperature. The output of unchanged amphetamine was most in man and

least in rabbits. The dose of amphetamine given to the human subjects was less than 1/100 of that given to the rats and rabbits.

p-Hydroxyamphetamine is a major metabolite in rats but not in man, rabbit and the dog; it was excreted in a conjugated form which was hydrolysed by heating the urine with an equal volume of 10N hydrochloric acid at 100° for 2 hr.

Benzyl methyl ketone was not found as such in any of the urines examined, but when human, rabbit or dog urine was heated as above with 10N hydrochloric acid, benzyl methyl ketone was produced. None was found in rat urine under the same conditions. A total of 2·4 g of (±)-amphetamine sulphate was fed during 3 days to 8 rabbits at the rate of 100 mg/rabbit/day. The urine from these rabbits was hydrolysed with 10N hydrochloric acid as above and then steam distilled. The cloudy steam-distillate was treated with 2,4-dinitrophenylhydrazine hydrochloride in ethanol (Brady's reagent) and the 2,4-dinitrophenylhydrazone of benzyl methyl ketone (m.p. and mixed m.p. 151°) was isolated (60 mg) and characterised. For quantitative estimations by isotope

TABLE 1. THE ELIMINATION OF ^{14}C AFTER ^{14}C-AMPHETAMINE SULPHATE IN VARIOUS MAMMALS

	Rat			Rabbit	Man	Dog
	(+)**	(−)	(±)	(±)	(±)	(±)
Dose (oral) of drug, mg/kg	10	10	10	10	0·07	5 (i.p.)*
Dose of ^{14}C, μC	2	10	10	15	7	20
No. of animals	3	3	2	2	3	1
^{14}C output	% of dose†					
In urine on Day 1	79	68	81	81	66	89
2	6	11	4	6	19	2
3	3	2	1	5	6	0·5
Total	88	81	86	92	91	91·5
In faeces on Day 1	2	3	3	5	—	—
2	1	1·5	2	1	—	—
3	0·25	0·1	0·5	1	—	—
Total	3·25	4·6	5·5	7	—	—
Total excretion	91	86	91	99	91	91·5

* The drug was administered intraperitoneally to this greyhound.
** Optical form of drug
† Average values, to nearest whole number in most experiments.

TABLE 2. THE URINARY EXCRETION OF VARIOUS METABOLITES OF AMPHETAMINE SULPHATE IN MAN AND OTHER MAMMALS
Dose of drug and ^{14}C and number of animals as in Table 1

	Rat			Rabbit	Man	Dog
	(−)*§	(−)	(±)	(±)	(±)	(±)
Metabolites found in urine in % of dose**	Days after dosing†					
	2	2	2	1	1	1
Amphetamine	12	17	13	4	30	38
p-Hydroxyamphetamine	48	63	60	7	3	7
Benzyl methyl ketone	0	0	0	22	3	2·5
1-Phenylpropan-2-ol	0	0	0	8	0	2
Benzoic acid	2	2	3	27	20	32
Total of above metabolites	62	82	76	68	56	82
^{14}C in urine‡	85	80	84	81	66	89

* Optical form of drug. ** Average values to nearest whole number.

† Isotope dilution was carried out on urine collected for 2 days after dosing in the rat, and one day for the other species.

‡ The urinary pH was about 7·5 in rats and the dog, about 8 in the rabbits and 6·5 in man.

§ We are grateful to Dr. S. Kaplan for the data on the (+)-isomer.

dilution, the ketone was counted as the semicarbazone (m.p. 197–198°). The amount of the ketone obtained in this way was high in the two rabbits examined (18 and 25% of the dose) but low in the three human subjects (1·3, 2·0 and 6·8%), and the dog (2·5%). The nature of the precursor of the ketone in the urine has not yet been elucidated but it does not appear to be the corresponding imine, benzyl methyl ketimine. A conjugated form of 1-phenylpropan-2-ol (shown to be a glucuronide with β-glucuronidase) was also found in rabbit and dog urine (Table 2) and there appeared to be traces in human urine, but none in rat urine. The alcohol was measured by reverse isotope dilution after acid hydrolysis of the urine and counted as the phenylurethane (m.p. 92°).

The total ^{14}C-benzoic acid in the urine of rabbit, man and dog after ^{14}C-amphetamine amounted to about 20–30% of the dose (Table 2) in 24 hr. This benzoic acid occurred mainly as hippuric acid (about 80% of the total) in man, rabbit and dog urine. Paper chromatography of the radioactive urine from man suggested that the other 20% of the benzoic acid occurred as benzoyl-glucuronide. No spot was found corresponding to free benzoic acid. A small amount (2–3% of the dose) of labelled benzoic acid occurred in rat urine, mainly as hippuric acid.

Table 2 shows that there may be a difference in metabolism between (−)- and (−)-amphetamine in the rat. Less *p*-hydroxyamphetamine is excreted in 48 hours after administration of the (+)-than after the (−)-isomer. In the individual animals the output of *p*-hydroxyamphetamine after the (+)-isomer was 44, 49 and 50% of the dose, after the (−)-isomer, 58, 65 and 67%, and after the (±)-isomer, 58 and 62%. This could suggest that either the (+)- is less readily hydroxylated than the (−)-isomer or that (+)-*p*-hydroxyamphetamine is metabolised rather more readily than the (−)-isomer. In the 48-hr urines from rats, Table 2 shows that there is more urinary ^{14}C unaccounted for in (+)-amphetamine urine than in (−)-amphetamine urine. This could suggest that there are other minor metabolites which have not yet been identified. Preliminary isotope dilution studies have been made on rabbit urine for *p*-hydroxybenzyl methyl ketone, phenylpropionic acid, phenylpyruvic acid, phenyl-lactic acid, cinnamic acid, 2-amino-1-phenylpropan-1-ol (β-hydroxy-amphetamine), phenylacetic acid, mandelic acid and *p*-hydroxybenzoic acid, but the results have been negative. Rat urine was similarly tested for phenyl-alanine, β-hydroxyamphetamine, phenylacetic acid and *p*-hydroxybenzoic acid with negative results. *N*-Methylamphetamine (methamphetamine) was looked for in human urine, again with negative results.

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The effects of hypotonicity on the degranulating action of Compound 48/80 on mast cells

SIR,—The range of compounds releasing histamine and producing degranulation of mast cells is so wide and chemically diverse that it has proved difficult to find a unified concept which will explain their mechanism of action. Uvnäs & Antonsson (1963) have suggested that the degranulation process may be initiated by different chemical reactions and that the main differences in action of the compounds lie in their activity in this initial "triggering process." The nature of this process and of the final common pathway for these reactions remains in doubt.

As a result of a study of the action of Compound 48/80 in hypotonic solutions on rat mesenteric mast cells, Norton (1954) has postulated that Compound 48/80 produces degranulation of mast cells by increasing the permeability of the outer cell membrane to extracellular ions and that the concentration of ions within the cell leads to osmotic rupture and the release of granules. Furthermore, Asboe-Hansen (1964) has suggested that one function of the mast cell mucopolysaccharides is to absorb an excess of tissue water and that degranulation, with the release of these mucopolysaccharides, occurs in response to such an excess. Against the view that degranulation is produced by osmotic rupture of the cell, it has been shown by cinephotomicrography and electron microscopy (Horsfield, 1965a,b) that degranulation is an active process and does not involve dissolution of the external cell membrane. In addition, the degranulation produced by the application of chemical reagents takes up to 20 min before completion whereas osmotic rupture following the application of distilled water is complete within a few seconds. In view of these various findings the effects of hypotonic solutions on the degranulating action of Compound 48/80 have been re-examined.

In these *in vitro* experiments biopsies of rat mesentery were taken using a metal spring clip with opposing loops at one end. The biopsies, still in the clips, were placed in test solutions for 20 min at room temperature. The specimens were then fixed in methanol for 30 min and the discs of mesentery put on slides and a few drops of 0·1% toluidine blue in 50% methanol applied. The preparations were ringed with petroleum jelly and cover slips applied. 300 mast cells were counted in each preparation and the % degranulated cells recorded. Experiments were made in duplicate and average values plotted.

In the control series the biopsies were placed in various concentrations of saline and in the test series 0·1 μg/ml of Compound 48/80 was added to each solution. Since Högberg & Uvnäs (1960) have shown that calcium ions are necessary for degranulation with Compound 48/80, 1 mmol/100 ml of calcium chloride was added to the saline.

The results for the control series of experiments are shown in Fig. 1A. It is evident that significant degranulation does not occur until the tonicity of the test solution falls below one half that of an isotonic solution. Therefore above this level no correction need be applied in this system for degranulation due to the hypotonicity of the test solution. The results of the test series of experiments are shown in Fig. 1B. It can be seen that when Compound 48/80 is present

the % of mast cells degranulated steadily increases as the test solution becomes more hypotonic over the range 1·0–0·5 × isotonic.

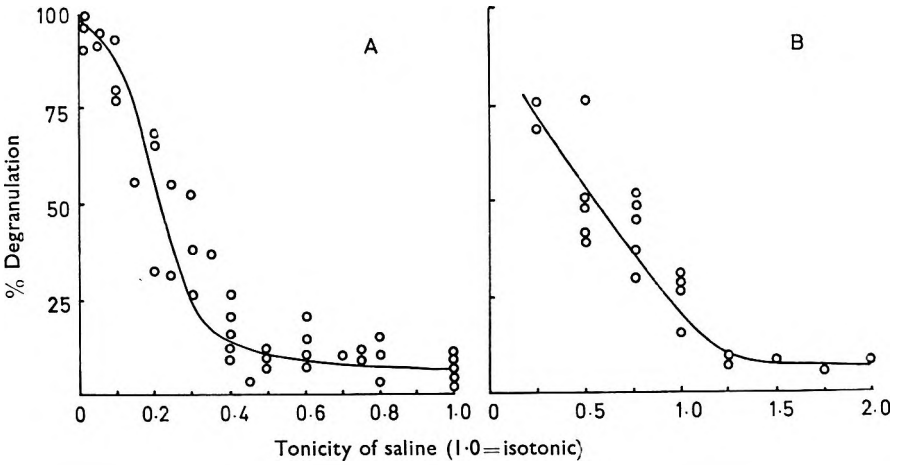

FIG. 1 The effect on rat mesenteric mast cells of hypotonic solutions of saline (A) and Compound 48/80 (B), 0·1 μg/ml, in the presence of varying tonicities of saline. In A degranulation is not increased until the tonicity reaches 0·4. Over the range 0–5—1·0 there is no significant difference between the amount of degranulation produced at each point. In B over the range 0·5—1·0 the amount of degranulation increases as the tonicity of test solution falls.

It can be concluded from these experiments that for degranulation to occur solely because of an excess of tissue water the tonicity of the extra-cellular fluid must fall to about half of normal (which seems unlikely under physiological conditions) and that lowering the tonicity, and thus the extracellular ion concentration, increases the degranulating action of Compound 48/80. It seems unlikely therefore that Compound 48/80 produces its effect by concentrating extracellular ions within the cell and thus producing osmotic rupture.

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Histamine and rat blood pressure

SIR,—In 1962, Beleslin reported that intravenous doses of histamine (10–50 μg/kg) produced in 75% of rats a fall of blood pressure which was followed by a pronounced rise. We have found that Wistar albino rats obtained from one colony showed this biphasic response to histamine when tested in the autumn but were unresponsive to histamine in the summer, whilst animals from another colony showed only a depressor response in the autumn and were also unresponsive in the summer. Thus, there is a colony difference, as well as a seasonal variation, in the response of Wistar rats to intravenous histamine.

Rats weighing 180–200 g were obtained from the Agricultural Research Council's Field Station, Compton (hereinafter called A.R.C. rats) and from the Wellcome Research Laboratories, Beckenham (hereinafter called BW rats). They were anaesthetised with urethane (1·5 g/kg) intraperitoneally and their tracheae were cannulated to reduce interference from excessive mucous secretion. Records of blood pressure were taken from the carotid artery with a Condon manometer; animals from both colonies had similar resting levels (95–100 mm Hg). Heparin (1,000 units/kg) was given intravenously and subsequent injections were made into the exposed femoral vein. During the months of September to January, the A.R.C. rats responded to histamine (10–50 μg/kg) with a fall of blood pressure of about 40 mm Hg which was always followed by a pronounced rise of about 20 mm Hg. At the same time of the year, the BW rats responded with a fall of blood pressure of about the same intensity, but no secondary rise followed. The secondary rise obtained in A.R.C. rats diminished with repeated doses of histamine and was restored when a single intravenous dose of noradrenaline (1–2 μg) was given 2–5 min before the next histamine dose. In fact, noradrenaline often markedly increased the secondary rise of blood pressure produced in A.R.C. rats by histamine early in some experiments, although it did not modify the initial fall of pressure. The secondary rise was greatly reduced by adrenalectomy, suggesting that in these rats histamine released relatively large amounts of noradrenaline and adrenaline from the adrenal medulla.

TABLE 1. BLOOD PRESSURE RESPONSES OF WISTAR RATS FROM TWO COLONIES TO INTRAVENOUS DOSES OF HISTAMINE AT DIFFERENT TIMES OF THE YEAR

Time of year	No. of rats	Threshold dose of histamine (μg/kg)	Response	
			A.R.C. rats	BW rats
Sept.–Jan.	51	10–50	Depressor followed by a secondary rise	Depressor only
Feb.–April	25	100–500	Slight depressor followed by a a secondary rise	Slight depressor only
May–Aug.	47	1000—5000	None	None

During February and March, the sensitivity of rats from both colonies to intravenous histamine decreased about ten-fold and only reduced depressor and secondary pressor responses were obtained in A.R.C. rats.

By April, all animals from both colonies were completely insensitive to histamine, doses as high as 5 mg/kg having no effect on the blood pressure. This insensitivity remained for the next 4 months and then reactivity suddenly returned (usually in late August), the difference in response of the rats from the two colonies again becoming prominent.

The present results showing that rats are relatively insensitive to histamine during the summer months may be linked with the seasonal variation in the response of rats to anaphylactic shock, experimental traumatic shock, and tourniquet shock, some colonies being resistant during the period from May to August each year (Ankier, Dawson, Karady & West, 1965). Furthermore, the secondary rise of blood pressure produced in A.R.C. rats by the injection of histamine and probably resulting from the release of catecholamines helps to explain why A.R.C. rats are more resistant to histamine liberators such as dextran than are BW rats (Ankier, Harris, Luscombe & West, 1965; Fearn & West, 1965). The importance of stating the time of year when experimental results are obtained is again stressed.

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meso-OO'-Succinylbis(β-methylcholine)

SIR,—Attention has been drawn (Lesser, 1961) to an apparently specific effect of *meso*-succinylbis(β-methylcholine), in that it produced a contracture in the innervated chick biventer cervicis preparation, whereas the optical enantiomorphs showed a reduction of twitch height without contracture. The material used was believed to be a mixture of the racemic and *meso-OO'*-succinylbis-(β-methylcholine iodides) (Clitherow, 1961).

Since the publication of that note, a sample of the *meso*-compound prepared by a specific synthesis has come to hand (Clitherow, personal communication) and has been tested. The results did not bear out the original observation. This compound not only had qualitatively the same action as the optical isomers but also had quantitatively an activity lying intermediate between theirs.

There are some indications that the effect previously observed may have been due to contamination of the commercially obtained 1-dimethylaminopropan-2-ol by 2-dimethylaminopropan-1-ol, which as a result, yielded some *OO'*-succinylbis(α-methylcholine). This is being further investigated.

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Contents

Letters to the Editor

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