TABLE II

Drug	Mean Time Taken to Reach Intolerable Pain (T ₁₀) (Minutes)	Standard Error of the Difference Between Any Two Means
(a) Pethidine (22.5 mg.)	13.4	0.83
(b) Pethidine: levallorphan (300 : 1)	15.1	
(c) " " (300 : 2)	14.8	
(d) Saline	11.4	

time taken to reach intolerable pain (T₁₀) was significantly longer than in the control experiments when saline was given. From the means it appears probable that the pethidine-levallorphan mixtures were more effective than pethidine in postponing intolerable pain.

Thermal Pain Threshold.—Thresholds were measured before, and 30, 60, and 90 minutes after the injection. The figures given in Table III are changes in the pricking-pain

TABLE III.—Changes in Thermal Pain Following Administration of Pethidine and Pethidine-Levallorphan Mixtures

Drug	Minutes after Injection		
	30	60	90
(a) Pethidine	+12.5	+ 5.6	+ 3.3
(b) Pethidine: levallorphan (300 : 1)	+19.8	+14.1	+10.1
(c) " " (300 : 2)	+13.2	+ 7.2	+ 3.7
(d) Saline	— 0.3	— 0.1	+ 0.7

threshold expressed as a percentage of the pre-injection threshold. The rise in threshold was greatest after 30 minutes; at 60 and 90 minutes the thresholds were returning towards the pre-injection values.

In the statistical analysis of these results it has been assumed that the random error of the pre-injection threshold measurements was the same as the error of the other measurements. All three drug injections significantly increased the threshold, while saline did not. A difference of more than 5.5 between any two drugs is significant at the 5% level. The mixture containing the smaller amount of levallorphan (b) is thus significantly more effective than pethidine and the other mixture (c).

Discussion

Both methods depend largely on the subject's reaction to pain, as well as on the pain sensation and the physical stimulus provoking it. The ischaemic pain method has the advantage that drugs are tested under conditions close to clinical situations; in practice conscious patients nearly always have a pain before they are given a drug to relieve it. The greater precision of the thermal pain threshold method is offset by its dependence on skin temperature (Whyte, 1951; Jackson, 1952), so that changes in blood flow and sweating caused by the drugs may affect the results. Greater apparent analgesia after one injection compared with another might thus be due to changes in the local blood flow rather than to an action in the brain.

The results show that the two pethidine-levallorphan mixtures relieve ischaemic pain more effectively than does pethidine. The mixtures are also no less effective than pethidine in raising the thermal pain threshold. Lasagna and Beecher (1954) have reported similar results using mixtures of morphine and nalorphine in patients with post-operative pain.

In the thermal pain experiments it was surprising that the mixture containing the smaller dose of levallorphan caused the greatest rise in threshold. If this is a real effect, and not due to chance, it might be explained in several ways. The lower dose of levallorphan might cause vasodilatation and/or sweating, and so raise the threshold, while with the higher dose the antagonism for pethidine becomes manifest. On the other hand, Yim *et al.* (1955) have shown in rabbits that levallorphan is a weak analgesic, and this suggests the possibility that the rise in threshold is due to an analgesic

action which is added to that of pethidine. This might be investigated further by using higher doses of suitable pethidine-levallorphan mixtures, and testing analgesia by a different method.

Costa and Bonnycastle (1955) found that, in rats, levallorphan antagonizes respiratory depression produced by pethidine more than analgesia. Our results show that in man the addition of levallorphan does not necessarily impair the analgesic action of pethidine. Whether the addition of levallorphan is of value in clinical practice can only be decided by a trial in patients under clinical conditions.

Summary

A comparison has been made between the analgesia produced by intravenous injections of (a) pethidine 22.5 mg., (b) pethidine 22.5 mg. + levallorphan 0.075 mg. (dose ratio 300 : 1), (c) pethidine 22.5 mg. + levallorphan 0.15 mg. (dose ratio 300 : 2), and (d) physiological saline solution. The effects of these drugs on ischaemic muscle pain and on the thermal pain threshold were measured.

The pethidine-levallorphan mixtures produced no less analgesia than did pethidine; the mixture containing the smaller amount of levallorphan—(b) above—produced significantly greater analgesia than pethidine. This unexpected result is discussed.

We are grateful to Dr. P. Armitage for statistical advice and for analysing the results, and to Dr. R. S. Stacey for helpful discussion and criticism. The drugs used were supplied by Roche Products Ltd., who also defrayed some of the expenses of the investigation; it was a pleasure to work with Dr. J. F. T. Allison, their Director of Clinical Research at the time the experiments were done.

REFERENCES

- Costa, P. J., and Bonnycastle, D. D. (1955). *J. Pharmacol.*, **113**, 310.
 Hardy, J. D., Wolff, H. G., and Goodell, H. (1940). *J. clin. Invest.*, **19**, 649.
 Hewer, A. J. H., and Keele, C. A. (1948). *Lancet*, **2**, 683.
 Jackson, H. (1952). *Brit. J. Pharmacol.*, **7**, 204.
 Lasagna, L., and Beecher, H. K. (1954). *J. Pharmacol.*, **112**, 356.
 Whyte, H. M. (1951). *Clin. Sci.*, **10**, 333.
 Yim, G. K. W., Keasling, H. H., Gross, E. G., and Mitchell, C. W. (1955). *J. Pharmacol.*, **115**, 96.

FATE OF BILE PIGMENT IN CHOLERA

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The rice-water stool of cholera is well known to every student of tropical medicine. It is believed to be due to the absence of bile pigment from the intestine caused by congestion and oedema of the mucous membrane of the biliary tract, and thus to obstruction of the flow of bile to the duodenum (Rogers, 1921). De and Chatterji (1935), on the other hand, suggested that this is brought about by the depression of the secretory function of the liver in cholera. It was felt that these explanations were not satisfactory, since we could not detect any obstruction to the biliary flow at necropsy on cholera patients who died in the stage of purging. We found that gentle pressure on the gall-bladder led to the escape of bile into the duodenum. Investigations were therefore undertaken to ascertain the fate of bile pigment in cholera patients.

Method and Material

Investigations were carried out on 52 cholera patients who were admitted into the infectious diseases ward of Nilratan Sircar Hospital during the epidemic of 1956. *Vibrio cholerae* (47 Inaba strain and 5 Ogawa strain) was isolated from the stools of all these patients. Stools were collected immediately after admission. Ten millilitres of blood was drawn from a vein (in some cases before any saline transfusion was given and in others after saline transfusion; in the latter cases the blood was drawn from the arm opposite to that into which saline was transfused); 2 ml. of it was mixed with oxalate powder to determine the packed cell volume and the erythrocyte count; 0.9 ml. was added to 0.1 ml. of 3.8% sodium citrate solution to measure the plasma haemoglobin. The rest was allowed to clot for the separation of serum to determine the serum bilirubin, for the thymol turbidity test, and to estimate the alkaline phosphatase. As most of the patients were admitted in the stage of anuria a close watch was kept and the first sample of urine was collected.

The serum bilirubin was estimated according to the method of Malloy and Evelyn (1937), the thymol turbidity by Shank and Hoagland's (1946) method, the plasma haemoglobin by Dacie's (1954) method, and the serum alkaline phosphatase by the method of Harrison (1949). The technique of MacLagan (1946) was used to determine the faecal urobilinogen. Urinary urobilinogen was detected by the method of Watson *et al.* (1944).

Result

A summary of our findings is given in the accompanying Table.

Summary of the Findings

	Faecal Urobilinogen (mg./100 ml.)	Serum Bilirubin (mg./100 ml.)	Packed Cell Volume (%)	Plasma Haemoglobin (mg./100 ml.)	Thymol Turbidity Test (Units)	Alkaline Phosphatase (K.A. Units)
On admission	0.08 (52)	0.81 (52)	54 (52)	6.1 (46)	3.5 (40)	8.6 (42)
24 hours later	27.3 (28)	0.58 (28)	45 (28)	3.5 (27)	Not done	Not done
7 days later	135.1 (42)	0.35 (46)	41 (46)	3.1 (42)	" "	" "

Figures in parentheses indicate the number of cases examined.

Faecal Urobilinogen.—The stools of 52 patients were examined soon after admission (usually six to eight hours after onset); 37 had no faecal urobilinogen and in 15 the pigment varied in concentration from 0.07 to 0.52 mg./100 ml. The samples of stool which gave a positive reaction had a yellowish tinge. The test thus appeared to be no more sensitive than could be detected with the naked eye. In most cholera patients the stool does not contain any substance which can react with Ehrlich's reagent. Since we did not perform the petroleum ether extraction of the stool we cannot be sure that the positive reaction with Ehrlich's reagent in the 15 cases was wholly due to faecal urobilinogen and not to some other substance. We do not think that the whole of this Ehrlich reacting substance is something other than urobilinogen, for if it were there is no reason why a similar substance was absent from the stools of the other 37 patients. We are therefore inclined to believe that a part, if not the whole, of this Ehrlich reacting substance is faecal urobilinogen. MacLagan pointed out that "the error introduced by omitting ether extraction is likely to be small" in comparison with other known errors that occur in the estimation of faecal urobilinogen. The pigment reappeared in the stools of all patients within 12 to 24 hours of admission to hospital. The average amount of pigment was 27.3 mg./100 ml. Seven days later this was 135.1 mg./100 g. (stool was formed by this time).

Urinary Urobilinogen.—Since at the time of admission these patients were anuric, the first sample of urine was obtained usually 12 to 24 hours after admission. The urine always gave a positive reaction with Ehrlich's reagent, but

the quantitative estimation was not possible because of the precipitation of albumin with the addition of the reagent, which is acid in reaction. The precipitate caused so much turbidity as to interfere with the colorimetric estimation.

Serum Bilirubin.—The average serum bilirubin of these patients on admission was 0.81 mg./100 ml. Since there was in every case some haemoconcentration, the actual amount when corrected for haemoconcentration was 0.63 mg./100 ml. Although this figure is within the normal range—namely, 0.2 to 0.8 mg./100 ml. (Whitby and Britton, 1953)—it is higher than what we found seven days later (0.35 mg./100 ml.), when there was no haemoconcentration. There is therefore a slight rise of serum bilirubin in cholera patients at the onset of their illness. The direct van den Bergh test was negative in all cases.

Haemoconcentration.—The degree of haemoconcentration on average was 22% as calculated from the P.C.V. on admission (54%) and a week later (41%). The plasma volume was restored to normal within two to four days, and by the seventh day the distribution of the body fluid was normal.

Plasma Haemoglobin.—At the time of admission the plasma haemoglobin was 6.1 mg./100 ml. The actual figure when corrected for haemoconcentration was 4.6 mg./100 ml. There was thus no increase in the plasma haemoglobin concentration of these patients. Seven days later this was 3.1 mg./100 ml.

Thymol Turbidity Test.—The average figure on admission was 3.5 units. This is within the normal range, which is 0 to 4.7 units by this method. There is therefore no evidence of any depression of hepatic function in cholera.

Serum Alkaline Phosphatase.—The average figure found on admission was 8.6 King-Armstrong units per 100 ml. of serum; the maximum was 12 units and the minimum 6 units. In seven cases the blood was collected before any saline transfusion, and in none of these was the figure more than 12 units. This finding, together with the negative direct van den Bergh reaction, is a point against the assumption that there is any obstruction to the flow of bile into the intestine.

Discussion

Faecal urobilinogen was found to be absent from the stools of most cholera patients, though in a small number it was present in a detectable quantity. But we cannot accept the usual explanation that it is due to obstruction of the flow of bile caused by the oedema and congestion of the mucous membrane of the biliary tract, for the following reasons. Firstly, because we could not detect any such obstruction in the cases of cholera which came from necropsy in the stage of purging. Secondly, the normal serum alkaline phosphatase level suggests that the flow of bile from the biliary tract into the duodenum is unhampered. The fact that the direct van den Bergh test was negative in these patients is a further point against this assumption. Indeed, it is difficult to envisage that a pathological process in which there is so much loss of fluid from the small intestine will permit the collection of so much fluid in the wall of the biliary tract as to cause oedematous swelling of the mucosa.

The presence of urinary urobilinogen in the sample of urine collected as soon as the anurial phase passed off indicates that urobilinogen must be present in the gut, for it is well known that urinary urobilinogen arises from the reabsorption of faecal urobilinogen from the gut (Muller, 1892). It is therefore reasonable to believe that bile pigment does flow into the intestine in cholera patients. In our opinion the reason for the failure to detect the pigment in the stool is due either to washing out (a cholera patient passes on an average 8 to 10 pints (4.4 to 5.7 litres) of liquid stool in 24 hours) or to dilution of the stool so that the test is not sensitive enough to detect the infinitesimal amount of this pigment present. Another possibility is that bilirubin is converted into some compound which does not react with Ehrlich's reagent. It is well known that faecal urobilinogen does not account for the whole of the bilirubin formed in the body, as a variable amount of bilirubin is converted into some unknown compound that does not

react with Ehrlich's reagent (Gray, 1953). Spectroscopic examination of cholera stool might show some such compound.

The presence of *E. coli* in the gut is necessary for the conversion of bilirubin into faecal urobilinogen (Baumgartel, 1950). The temporary alteration of the intestinal flora (since the *E. coli* population becomes very meagre in the early stage of cholera) may be responsible for the failure to convert bilirubin into urobilinogen. Thus Sborov *et al.* (1951) found that in patients treated with chlortetracycline urobilinogen disappeared from the stool.

Although it is hazardous to draw any conclusion from a single liver-function test, a normal thymol turbidity test and the absence of an abnormal amount of urobilinogen in the urine of cholera patients suggest that there is probably no gross dysfunction of liver in this disease. This is further supported by the fact that the histological appearance of liver in cholera patients does not show any gross abnormality.

What, then, is the cause of the small rise of serum bilirubin that we observed in these patients in the early stages? Haemolytic mechanism has been suggested in cholera (De *et al.*, 1952, 1954). But our finding of serum bilirubin was within the normal range, and as we could not detect any rise in the plasma haemoglobin level we can definitely exclude the role of haemolysis. In fact, the early enthusiastic papers of the above workers are followed by a more cautious report, where they recorded a much smaller percentage of patients showing signs of haemolysis than what they had found before (De, 1954). The haemoconcentration that occurs in cholera entails a greater osmotic work for the liver cells to pick up bilirubin from the blood for secretion into the bile capillary. This may be the cause of diminished secretion of bile and the slight rise of serum bilirubin concentration that we observed in cholera patients.

Summary

Tests of the stools of cholera patients in the stage of purging showed that in most of them there was no detectable amount of urobilinogen.

The serum alkaline phosphatase level and the thymol turbidity of these patients remain within the normal range.

Evidence is given which suggests that there is no obstruction to the flow of bile into the gut in cholera, and some possible explanations for the absence of faecal urobilinogen in these patients are discussed.

Although there is a slight rise in serum bilirubin in these patients there is no evidence of any haemolysis, nor is there any evidence of the depression of hepatic function.

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REFERENCES

- Baumgartel, T. (1950). Quoted by C. H. Gray (1953) in *The Bile Pigments*, p. 68.
 Dacie, J. V. (1954). *The Haemolytic Anaemias*, p. 506. Churchill, London.
 De, M. N., and Chatterji, K. D. (1935). *Bacteriology*, p. 520. Statesman Press, Calcutta.
 De, S. N. (1954). Technical Report of the Scientific Advisory Board for the Year 1954, p. 294. Indian Council of Medical Research, New Delhi.
 — Sengupta, K. P., and Chanda, N. N. (1952). *Brit. med. J.*, 2, 22.
 — (1954). *Lancet*, 1, 807.
 Gray, C. H. (1953). *The Bile Pigments*, p. 47. Methuen.
 Harrison, G. A. (1949). *Chemical Methods in Clinical Medicine*, 3rd ed., p. 365. Churchill, London.
 MacLagan, N. F. (1946). *Brit. J. exp. Path.*, 27, 190.
 Malloy, H. T., and Evelyn, K. A. (1937). *J. biol. Chem.*, 119, 481.
 Muller, F. V. (1892). Quoted by C. J. Watson in *Harvey Lectures*, 1950, 44, 41.
 Rogers, L. (1921). *Bowel Diseases in the Tropics*, p. 79. Henry Frowde; Hodder and Stoughton, London.
 Sborov, V. M., Jay, A. R., and Watson, C. J. (1951). *J. Lab. clin. Med.*, 37, 52.
 Shank, R. E., and Hoagland, C. L. (1946). *J. biol. Chem.*, 162, 133.
 Watson, C. J., Schwartz, S., Sborov, V., and Berti, E. (1944). *Amer. J. clin. Path.*, 14, 605.
 Whitby, L. E. H., and Britton, C. J. C. (1953). *Disorders of Blood*, 7th ed., p. 729. Churchill, London.

Medical Memoranda

Colporrhaphy During the Puerperium

It is well known that secondary repair of unhealed vaginal lacerations during the puerperium is a satisfactory procedure, but the advantages of deliberate formal perineorrhaphy have not attracted attention. In some cases this operation is urgently required, and from the patient's point of view it may be done most conveniently during the puerperium. At such a time the difficult arrangements for supervision of an existing family have been made, and a later visit to hospital will be more difficult to arrange as the family grows larger. The demands of a family are imperious, and most women will tolerate great discomfort for many years rather than forsake their maternal duties for a few weeks. From a national standpoint there is clearly economy of hospital beds and expense if a confinement and operation can be combined at a single admission and without additional days spent in hospital.

PRESENT SERIES

A short account is given of 46 patients upon whom formal perineal repair during the puerperium was practised during the past eight years. Their ages varied from 21 to 44 years, and the operation was done a few days (mostly four or five days) after delivery. In these days of restricted admission to maternity hospitals relatively few multiparae are delivered in hospital. This series is composed almost entirely of women referred for some abnormality to the antenatal and subfertility clinics. The principal reasons for consultation were as follows: (1) Eight women presented with prolapse, with actual protrusion of the cervix or of a rectocele or cystocele early in pregnancy. Pessary support was necessary in five of these cases. (2) Twelve women were referred from the subfertility clinic on account of multiple miscarriages (two to five miscarriages). (3) Sixteen women complained chiefly of urinary incontinence dating from a previous delivery and always worse during pregnancy. The following is a typical example.

Case 1.—A gravida 2, para. 1, aged 21, had had her first baby 18 months previously, delivered with forceps. She had had distressing urinary incontinence ever since. After delivery the perineum was seen to be very inadequate, with marked descent of the anterior vaginal wall. Simple posterior colporrhaphy on the fourth day after delivery cured her incontinence. Six weeks after delivery the perineum was sound and the bulge of the anterior vaginal wall was no longer seen.

In 25 cases there was complaint of pelvic discomfort and dragging pain after walking or standing, either alone or associated with other symptoms. In these cases the vulva is usually relaxed and gaping, often after a single confinement. The following example is typical.

Case 2.—A gravida 2, para 1, aged 26, had a very deficient perineum after two normal confinements, with constant backache, and some stress incontinence appeared during pregnancy. A simple posterior colporrhaphy was done on the ninth day after delivery. At post-natal examination seven weeks later she said her backache had gone and bladder control was normal. She added that her whole outlook upon life had improved since her operation; she felt full of energy and her husband had remarked upon her greatly improved temper.

After delivery the perineal tissues are remarkably supple and elastic. At operation dissection is easy, the fascial planes separate cleanly, and the levators can be brought together without tension. Healing proceeds rapidly; patients can get up on the fifth day after operation and should be ready to go home three to four days later—that is, departure from hospital is not delayed. Early ambulation does not seem to interfere with a good final result. No attempt has been made to carry out any elaborate procedure, and anterior colporrhaphy with support of the urethral angle has been performed once only. Nevertheless in 16 cases with marked stress incontinence dating from before the