proteins, but also in the toxins produced by them (Van Loghem and de Moor, 1938; Felsenfeld, 1960). Further, Tanamal *et al.* (1958) have shown that animals immunized with routine cholera vaccines are not satisfactorily protected against El Tor vibrios. Mass immunization with routine cholera vaccine should therefore be regarded of doubtful value as a measure against outbreaks of paracholera in epidemic form.

Summary

All the 130 strains of vibrios isolated in the 1961 cholera-like epidemic in Hong Kong have been found to belong to the El Tor type by haemolytic and phage-sensitivity tests. The importance of confirmatory tests like phage-susceptibility characteristics for the precise identification of the O group I vibrios has been discussed. The phage-susceptibility test is specially recommended in the case of strains isolated early in an outbreak.

The pattern of change in the geographical distribution of choleraic disease due to El Tor vibrios has been discussed and the consequent importance of precise bacteriological characterization of fresh outbreaks emphasized. In view of the differences in chemical composition and toxigenicity of *V. cholerae* and El Tor vibrios mass immunization with routine cholera inoculations should be regarded as of doubtful value.

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TWENTY-ONE UNCOMMON CASES OF POISONING

BY

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Deaths from poisoning number about 5,000 a year; carbon monoxide, the barbiturates, and aspirin account for most of these cases. The details of other poisonings are rarely of sufficient interest to warrant publication, because they involve such old favourites as arsenic trioxide, strychnine, morphine, nicotine, yellow phosphorus, or the halogenated hydrocarbons. Even when an unusual case involving death from a suicidal or accidental overdose of a new drug is encountered it is often not of enough scientific interest to publish.

Details of uncommon cases are nevertheless of interest to the toxicologist and for the purposes of statistical analysis; the following cases (Tables I and II) have been selected, therefore, from the ones submitted to this laboratory over the past 10 years for the analyses of which I have been responsible. The laboratory serves the whole of Yorkshire, an area of about 3,500,000 acres (1,416,400 hectares). Poisoning, or allergic response (Cases 15 and 16), was in all cases implicated either from the analyses or from circumstantial evidence.

TABLE I.—General Analysis of Cases

Case No.	Poison	Source	Quantity	Sex and Age	Weight		Time between Ingestion and Death
					st. lb.	kg.	
1	Dichloral phenazone	Wellborm	60–100 tablets	M 65	12	76.2	<3 hours
2	Orphenadrine	Elimit tablets	Not known	F 34	8	50.8	5½ "
3	Imipramine	Tofranil	60 tablets, size not known	F 22	9	57.2	<5 "
4	Thioridazine	Melleril	20, 100-mg. tablets	M 61	9 7	60.3	2½ "
5	Ethyl-2: 4-dichloro-phenoxy acetate	Dicotox Extra	3–4 oz. (60–90 g.)	F 49	8	50.8	About 2½ days
6	Insulin	Protamine zinc insulin	Not known. ? 1,200 units	M 53			At least 16 hours
7	"	Soluble insulin 40 units/ml.	Not known	M 22	10	63.5	About 13 hours
8	"	Protamine zinc insulin	? 800 units	M 48			Not known
9	"	Insulin zinc suspension 80 units/ml.	Injections of about 1 ml. on Mon., Tues., Wed., Thurs.	F 19			Admitted to hospital in coma on Fri. 4.30 a.m.
10	Hydrogen fluoride	Movol iron-mould remover	1.5 g. hydrogen fluoride	F 27			About 6½ hours
11	Phalloidin	A. phalloides	Not known	F 72	8 7	54.0	34 hours
12	"	"	"	M 45	11	69.9	2½ days
13	Adrenaline	Accidental injection of nebula adrenaline and atropine co. in hospital	2 ml. = 9 mg. adrenaline	M 37	12	76.2	30 minutes
14	Aminophylline	Corosidine tablets	Not known	F 1½	1 6	9.1	About 17 hours
15	Pollen vaccine	Combined pollen vaccine—strong	1.2 ml.	M 50	—	—	A few minutes
16	" "	Pollen extract of timothy grass and cocksfoot	200 units	F 52	—	—	Immediate collapse
17	Paraldehyde	"	2 dr. (8 g.) in water	M 32			43 hours
18	Glutethimide	Doriden	? 60 tablets	F 60	12	76.2	<12 hours
19	"	"	? 28 "	M 39	10	63.5	Missing several days
20	"	"	? 30 "	F 56	10	63.5	Found dead
21	"	"	? 36 "	F 50	—	—	" "

Discussion

This series includes the first recorded deaths from "welldorm," "elimit," and ethyl-2:4-dichlorophenoxy acetate, and the second from thioridazine ("melleril") (Enticknap and Gordon, 1961). In the case of welldorm poisoning the manufacturers inform me that vomiting usually follows ingestion of an overdose; it may be that the simultaneous ingestion of about 4 oz. (114 ml.) of rum suppressed it in this case. In the death from elimit the enteric coating of the tablet delayed absorption and made stomach lavage ineffective.

All the deaths from insulin poisoning occurred in diabetics who were found in circumstances which

suggested that they had injected themselves with massive doses. As such they were distinguished from accidental hypoglycaemia, although the analytical and pathological findings were essentially negative. These cases, together with a case previously reported (Birkinshaw *et al.*, 1958), make four deaths in a population of about five million in four years. The use of insulin in an unsuccessful attempt to procure an abortion (Case 9) was the subject of a criminal charge. The victim, a girl of 19, was not a diabetic, nor was her father, who pleaded guilty to the offence.

Elliott *et al.* (1961) say that no cases of *Amanita phalloides* poisoning have been reported in the English literature since 1949: the present paper indicates that at least two have occurred. The chemical approach to the detection of phalloidin in the intestinal contents has been described elsewhere (Curry, 1960). The cases of glutethimide ("doriden") poisoning are reported because it is interesting to note that, although these are only four in number, three of the subjects chose additional methods of death (plastic bag over face, drowning, and cut throat and wrists).

One outstanding point arises from a consideration of the chemical analyses (Table III); this is the extremely low blood concentrations of orphenadrine, imipramine, and thioridazine in cases where death followed the ingestion of a large overdose. These cases emphasize the great difficulties of the clinical biochemist in detecting drugs in small volumes of blood, but the toxicologist will note that the concentration in the liver was in all cases 5 mg./100 g. or more. The results also add to the list of drugs whose action is mainly on the brain but which are not found there in the highest concentration. Normal extraction procedures for imipramine and thioridazine were found to be very inefficient, and a five-minute hydrolysis in 40% hydrochloric acid at 100° C. was necessary to free the bound drug.

TABLE II.—Clinical and Post-mortem Findings

Case No.	Clinical Findings	Post-mortem Findings	Pathologist
1	"Drunk"; on to coma	Marked oedema of lungs: intense congestion of stomach mucosa. Mass of powder in stomach	Dr. D. MacKinnon
2	Confused; grand-mal seizures; dilated pupils; B.P. 110/80	Congestion of lungs: no other obvious finding	Dr. G. A. C. Summers
3	Found dead	No obvious findings related to poisoning	Dr. G. A. C. Summers
4	Given a stomach lavage immediately, but collapsed and died suddenly 2 hours later	Oedematous lungs: no other obvious lesion related to poisoning	Dr. J. Eastwood
5	Semicoma	Kidney: degenerate convoluted tubules. Protein in glomerular spaces; a little fatty degeneration. No other lesions	Dr. D. E. Price
6	Found in coma; died shortly afterwards in hospital	Terminal pulmonary oedema	Dr. R. E. Potts
7	Coma; admitted to hospital; given dextrose, sent home; relapsed back into coma and died	No obvious finding	Dr. D. E. Price
8	Found dead. Missing several days	Congested lungs. Other organs normal	Dr. J. P. S. Whitehead
9	Required intravenous glucose. Relapsed back into coma despite oral glucose 18 hours after admission. Uneventful recovery thereafter	—	Clinicians: Dr. P. Goodwin, Dr. L. W. Smith
10	Conscious, vomited blood: uncooperative; later collapsed and died	No gross damage related to poisoning	Dr. J. V. Wilson
11	Diarrhoea; vomiting	Necrotic mucosa of stomach, duodenum, and intestines. Acute yellow atrophy of liver showing severe fatty changes. Fatty degeneration in heart. Marked necrosis of proximal tubules in kidneys	Dr. P. Roche
12	Diarrhoea; vomiting; and thirst after symptomless period of 12-15 hours	Acute liver and kidney damage without demonstrable bacteriological cause	Dr. D. A. Slade
13	Collapse	Generalized venous congestion	Dr. D. E. Price
14	Thirsty; very restless; vomited dark-brown fluid	Non-contributory	Prof. C. J. Polson
15	Collapse within a few minutes	No obvious finding	Prof. C. J. Polson
16	Immediate collapse	Gross capillary engorgement in cortex of brain, lungs, and kidneys. Acute pulmonary oedema. Asphyxial pattern	Dr. I. S. Stewart
17	Chronic tuberculosis patient	Bronchopneumonia as sequel to corrosion to upper air passages	Dr. G. Forbes
18	Found dead; plastic bag over face	Nil significant, respiratory failure	Dr. W. L. Rose
19	Found dead in a stream; missing several days	Exposure	Dr. D. E. Price
20	Found dead; drowned in a bath	Drowning	Dr. A. J. Shillitoe
21	Found dead, cut throat and wrists	Haemorrhage	Dr. K. Froome

TABLE III.—Results of Chemical Analyses

Case No.	Chemical Analyses
1	30 tablets in stomach. Blood chloral 45 mg./100 ml. Liver 71 mg./100 g. Brain 8.25 mg./100 g. (uncorrected for recovery losses approximately 50%). Blood alcohol 50 mg./100 g.
2	950 mg. (19 tablets) in small intestines. Liver 5 mg./100 g. Blood less than 0.04 mg./100 ml.
3	Gastro-intestinal tract 27 mg. Blood 0.28 mg./100 ml. Liver 21 mg./100 g. Kidney 3.8 mg./100 g. Brain 2.9 mg./100 g. Unchanged drug recovered
4	Stomach contents 0.27 g. Blood 0.18 mg./100 g. Liver 7.8 mg./100 g. Undetectable in urine. Unchanged drug recovered
5	2:4-Dichlorophenoxy acetic acid recovered from tissues. Heart blood 40 mg./100 g. Peripheral blood 26 mg./100 g. Liver 15 mg./100 g. Brain 4.5 mg./100 g. Urine 39 mg./100 g.
6	Non-contributory. No insulin detectable in suspected injection sites
7	No insulin detectable in injection sites. Blood and C.S.F. levels all less than 40 mg./100 g. of glucose. Urine 510 mg./100 ml. glucose
8	No insulin detectable in injection sites in antecubital fossa suspected intravenous injection. Insulin isolated from hypodermic needles. Blood badly decomposed
9	Confined to showing presence of insulin in hypodermic syringe and needles
10	Nil detectable in stomach contents. Liver fluoride 165 µg./100 g. Test of Gettler and Ellerbrook (1939) on blood was positive
11	Extracts of mushrooms in colon examined by paper chromatography: eluates killed mice
12	Non-contributory. Exclusion of other poisons. No effort made to isolate phalloidin
13	Papaverine and possible traces of adrenaline isolated from injection sites
14	Phenobarbitone and papaverine isolated from intestines. Phenobarbitone isolated from liver. Ingestion of tablets shown
15	Non-contributory except to show absence of other poisons
16	
17	Paraldehyde was an old sample, contained 40% of glacial acetic acid plus excess oxidants
18	4.12 g. in stomach. Blood 2.3 mg./100 g. Liver 10 mg./100 g.
19	Blood 1 mg./100 g. Liver 1 mg./100 g.
20	3.95 g. in stomach contents. Liver 6.8 mg./100 g.
21	Stomach 225 mg. Duodenum contents 30 mg. Blood 1 mg./100 g. Liver 1.76 mg./100 g.

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Preliminary Communications

Copper Deficiency in Rats Fed upon Meat

The severe skeletal lesions which occur in young rats fed upon meat, without supplements of calcium, have already been described (Scott and Scott, 1960; Moore and Sharman, 1960). Further experiments have indicated another consistent abnormality, marked hypochromotrichia, in those parts of the piebald rat in which the fur is normally pigmented. Thus the fur of the head, back, and shoulders became light grey instead of black. A third lesion was anaemia, which was observed in most of the animals.

Clear evidence, to be reported later, has now shown that the effect of a meat diet upon the bones depends upon the age of the rats when the meat diet is started. Weanlings invariably develop severe lesions, usually including fractures, within a few weeks. In contrast adult animals can subsist for long periods on the same diet without sustaining skeletal lesions of any great severity. Both young and adult rats, however, become grey when they are fed solely upon meat. In adults the greyness was prevented by the addition to the diet of 0.5% of calcium carbonate. In young rats, however, this supplement was only partially effective against hypochromotrichia, although skeletal lesions were completely prevented. Anaemia, in parallel with hypochromotrichia, was reduced in severity, but not completely prevented, in young rats which were given calcium supplements.

Crude ferrous carbonate, when added to the meat at the high level of 2.5%, completely prevented both hypochromotrichia and anaemia, irrespective of the calcium intake. In contrast this addition of iron to the diet intensified the skeletal lesions in young rats not given calcium. It appeared at one point of the investigation, therefore, that both the hypochromotrichia and anaemia might be due to deficiency of iron in the meat, or the poor absorption of its iron contents by the rat. In view of the beneficial action of calcium against these abnormalities it appeared that calcium can influence the metabolism of iron. For this latter conclusion there is indeed ample further evidence.

Previous work on achromotrichia or hypochromotrichia in rats, however, has never implicated iron deficiency among the possible causes. Deficiencies of pantothenic and *p*-aminobenzoic acids have been shown to cause greyness, but these substances could certainly not have been present as impurities of iron carbonate even in its crudest form. Copper, however, is a well-known contaminant of iron salts, and its deficiency has

already been found to cause hypochromotrichia. Thus Hundley (1950) has shown that black rats become grey when they are fed upon purified diets deficient in copper, and that the greyness can be prevented by the addition of as little as 2.5 µg. of copper per g. of diet.

The suspicion therefore arose that the prevention of achromotrichia by crude ferrous carbonate might be due to copper present as impurity. The main purpose of this article is to describe a key experiment in which rats were given meat without mineral supplements or with supplements of crude iron, or pure iron, or copper.

THE EXPERIMENT

The animals used were female piebalds, mean body weight 114 g. They were all given a basal diet of minced raw beefsteak (skeletal muscle with attached adipose tissues) found by analysis to contain about 17% of fat. Doses of vitamins A and D were allowed, but it was thought that the meat contained adequate amounts of other vitamins. Four groups, each of six rats, were made up as follows.

Group 1: Minced steak only.

Group 2: Minced steak, with the addition of 2.5% of crude ferrous carbonate.

Group 3: Minced steak, with supplements of 2 mg. of iron per rat per day, as pure ferrous ammonium sulphate.

Group 4: Minced steak, with the addition of 10 µg. of copper per g. of steak, as copper sulphate.

The skeletal development of the rats at the start of the experiment was sufficiently mature to exclude severe lesions, and all the animals survived in apparently good health for 83 days, when the experiment was ended. Differences in the pigmentation of the animals appeared after about 28 days, and became more obvious as time advanced. Depigmentation was first seen at the periphery of the black parts, with greying at the sides of the shoulders and at the posterior end of the back. In many animals the hair on the crown of the head remained black long after other areas had become grey, with a sharp line of demarcation. Eventually most of the rats not given effective supplements became completely grey in those areas which are normally black.

Clear-cut differences were found between the incidences of hypochromotrichia in the different groups. All the rats which were undosed or were dosed with pure ferrous ammonium sulphate showed depigmentation. In contrast all those rats given a high intake of crude iron carbonate or small doses of copper remained normally pigmented. Fig. 1 shows the head of a typical rat of group 1 given no supplement. The marked depigmentation may be compared with the normal pigmentation of a rat dosed with copper (Fig. 2).

Other differences between the groups are summarized in the Table. It will be seen that mean blood haemoglobin was higher in the two groups which received either copper sulphate or impure ferrous carbonate than in the group which received the pure ferrous ammonium sulphate salt or no supplement. Moreover, weight increases with supplements of copper sulphate were greater than when no supplement or the pure ferrous salt was given. Statistical tests between group 1 (no supplement) and group 4 (CuSO_4) indicated that the difference was significant ($0.02 < P < 0.05$). On the other hand, weight gains were much lower in the rats given 2.5% of ferrous carbonate than in the group with no