A COMPARATIVE *IN VITRO* EVALUATION OF A NEW BISMUTH SALT BISMUTH ALUMINATE

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Received June 17, 1957

Antacids commonly used to treat peptic ulcer are compared for their ability to neutralise acid and inactivate pepsin. On this basis a new bismuth salt, bismuth aluminate is compared with ten other commonly employed substances and found to be the most effective agent. All preparations are grouped according to their antacid and antipeptic properties.

The numerous methods of *in vitro* evaluation of substances indicated in the treatment of peptic ulcer are almost excessively concerned with the neutralisation of hydrochloric acid[1–4]. The effect of substances upon the proteolytic enzyme content of gastric juice which may have an important bearing upon cause of ulcer pain and the persistent nature of the lesion are usually disregarded. It is hard to justify the pre-eminence given to the role of acid and the neglect of proteolytic enzymes. Ulcer pain is usually attributed to acidity or to abnormal motility of the gastro-duodenal area. If these are the only factors of importance then it is difficult to explain the action of substances which are neither antacids nor anti-spasmodics. The older bismuth salts such as the carbonate and sub-nitrate fall into this group, and yet they are capable of relieving the pain of peptic ulcer; they can, however, inactivate pepsin[5,6] regardless of the pH of the medium. Furthermore, in support of this hypothesis, ulcers occur in the absence of an excess of acid but not in the absence of active pepsin. In the normal stomach the HCl–pepsin complex does not attack the gastric mucosa but in the presence of peptic ulcer the mucosal defences are broken down and auto-digestion of the ulcer base may be the cause of its persistent nature. An important factor in the development of ulceration in the stomach of rats the pylorus of which has been tied was a gastric juice of high peptic activity[7].

The aim of ulcer therapy is said to be to restore gastric contents to pH 2–4 for as long a period as possible. Many simple antacids exceed this requirement by raising the hydrogen ion concentration above pH 5: by so doing these substances inactivate pepsin but may also give additional stimulus to acid secretion[8]. The ideal therapeutic agent will maintain the gastric contents at pH 2–4, and at the same time inactivate pepsin.

The purpose of this report is to compare the properties of a new bismuth salt, bismuth aluminate $Bi_2(Al_2O_4)_3 10H_2O$ with a selection of those substances commonly employed in peptic ulcer therapy. Bismuth aluminate contains approximately 35 per cent bismuth by weight in the dry form. It was tested in this form and also as a cream containing 19 per cent of the salt by weight.

METHODS

Brindle[1] defined the neutralisation value of a compound as the weight required to neutralise 100 ml. of 0·05N HCl. This is determined by adding a measured quantity to 100 ml. of 0·05N HCl at 38° and continuously stirring. The amount used should be such that there will be excess of acid. After 4 hours the mixture is filtered, the filter paper washed and the residual amount of acid determined. An amount of the compound equivalent to 20 per cent excess over the neutralisation value is then added to 100 ml. of artificial gastric juice at 38° and the pH recorded throughout by electrodes immersed in the mix ture which is continuously stirred. The pH increase at given times is recorded and the amount of HCl neutralised calculated by reference to a standard graph (Fig. 1).

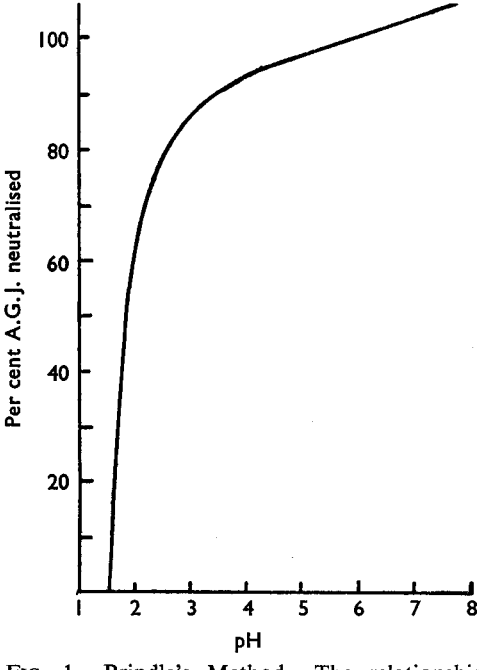

FIG. 1. Brindle's Method. The relationship of pH to the percentage of artificial gastric juice neutralised.

An experimental method was also devised in our own laboratory from the data of Armstrong and Martin[2] using the artificial gastric juice recommended by Brindle which contains 150 mg. each of pepsin, peptone and sodium chloride, and 0·05N HCl to 100 ml. adjusted to pH 1·4–1·5 at 37°. To 150 ml. of artificial gastric juice in an artificial stomach (Fig. 2) was added the dose, commonly recommended in therapy, of the preparation under test. The pH was recorded continuously and fresh artificial gastric juice allowed to drip in at 1·5 ml./minute. After each 20 minute period up to 100 minutes, 30 ml. of the mixture was removed and tested for pepsin.

Pepsin was determined by modification of the method of West, Ellis and Scott[9]. This is based upon the ability of pepsin to cause aggregation of casein particles in fresh homogenised milk. The reagents used are: acetate buffer pH 4·9 (sodium hydroxide 4·2 g., glacial acetic acid 9·2 ml., distilled water to 100·0 ml.); milk buffer mixture (equal parts of fresh homogenised milk and acetate buffer).

Before the test all reagents and the test solution are brought to 13° in a cold water bath. In a test tube are placed 2 ml. of acetate buffer, 1 ml. of distilled water and 1 ml. of test solution; to this mixture is added 1 ml. of milk buffer and a stop watch started. The test tube is shaken

and at the first appearance of aggregated particles in the film on the sides of the tube above the mixture the watch is stopped and the time noted. Each test solution is investigated at least twice and a mean precipitation time calculated. Preliminary tests indicated that if the time for the aggregation of casein particles was greater than 40 seconds, the experimental error in repeating the tests was less than 3 per cent.

Before investigating therapeutic substances, calibrating experiments to relate the time for casein precipitation to the concentration of pepsin in solutions of known strength were made. Samples of artificial gastric juice with a pepsin concentration from 25 to 300 mg./100 ml., and containing 150 mg. peptone and NaCl in 0·05 HCl were diluted 1 in 16 with distilled water and 1 ml. of diluted mixture was used as the test solution in the method described above.

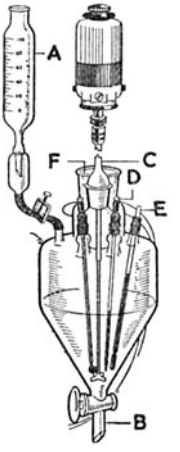

FIG. 2. The artificial stomach used for the second method of assessment devised in our laboratories.

KEY—A, measuring cylinder allowing intermittent addition of fresh artificial gastric juice; B, tap allowing intermittent removal of the mixture; C, constant stirrer; D, electrodes constantly registering pH; E, thermometer allowing control of temperature; F, wide necked opening for addition of substance under test. Heating is by a thermostatically controlled cuff which fits round the apparatus.

Solutions were tested under a number of conditions. It was found that samples of freshly homogenised milk did not vary significantly in casein concentration; that temperature exerted a marked effect upon the rate of precipitation, and that there was some variation in the potency of artificial gastric juice after storage. Although the actual precipitation time for a test solution was inconsistant, the logarithmic relation of pepsin concentration to time of precipitation remained constant being a straight line, the slope of which was always 1·12. From such a graph it was calculated that:

$$\log \text{pepsin concentration } a = \log \text{pepsin } 150 - (\log \text{precipitation time } a - \log \text{precipitation time } 150) \times 1{\cdot}12$$

where a = is the solution of unknown pepsin; 150 solution = solution containing 150 mg. per cent pepsin. (This solution was selected as standard since it is the artificial gastric juice used in each experiment.)

RESULTS

Brindle's Method

The results are shown in Table I. In terms of acid neutralisation, bismuth aluminate compares favourably with other substances and in the dry state is far superior to dry aluminium hydroxide the potency of which varies considerably from sample to sample. Table II shows the effect

TABLE I

BRINDLE'S TESTS. RESULTS

Substance	Neutralisation value	Change in pH and acid neutralised by adding 20 per cent excess over neutralisation value to 100 ml. A.G.J. at 38° C.							
		After 10 mins.		After 20 mins.		After 30 mins.		After 60 mins.	
		pH	HCl neutralised per cent	pH	HCl neutralised per cent	pH	HCl neutralised per cent	pH	HCl neutralised per cent
Bismuth carbonate	1·38 g.	0·15	30	0·18	33	0·18	33	0·25	41
Bismuth subnitrate	8·3 g.	0·42	56	0·45	58	0·42	56	0·40	54
*Aluminium hydroxide gel	8·707 g.	1·4	88	2·14	95	2·32	96	2·4	98
*Dried aluminium hydroxide gel (A)	0·1829 g.	0·17	30	0·24	40	0·32	49	0·45	60
*Dried aluminium hydroxide supplied as B.P.C. (B)	1·144 g.	0·98	81	1·76	91	1·93	93	1·98	94
Dried aluminium hydroxide	0·420 g.	0·46	51	0·50	55	0·61	61	0·74	65
*Magnesium trisilicate	0·435 g.	0·5	62·5	1·02	82·5	1·52	90	3·36	100
Bismuth aluminate powder	0·395 g. (0·139 g. Bi)	0·69	65	0·94	70	1·1	76	1·47	85
Bismuth aluminate cream	5·3 g. (0·367 g. Bi)	0·22	35	0·31	45	0·42	50	0·52	60

* Results taken from Brindle's original paper.

TABLE II

Substance	Neutralisation value	Change in pH and acid neutralised by adding therapeutic dose of bismuth aluminate to 100 ml. A.G.J. at 38° C.							
		After 10 mins.		After 20 mins.		After 30 mins.		After 60 mins.	
		pH	HCl neutralised per cent	pH	HCl neutralised per cent	pH	HCl neutralised per cent	pH	HCl neutralised per cent
Bismuth aluminate powder	2·01 g. (0·7 g. Bi)	2·21	82	2·34	88	2·46	89	2·62	90
Bismuth aluminate cream	10·1 g. (0·7 g. Bi)	0·63	64	0·76	66	0·86	71	1·12	82

of therapeutic doses of bismuth aluminate, virtually all the acid is neutralised within 10 minutes and the final pH (4·09, powder; 2·57, cream) is within the accepted desirable range.

These results also indicate the relative efficiency of the bismuth salts tested, in terms of acid neutralisation. This comparison is perhaps best

TABLE III

ANTACID POWER OF 2 G. OF BISMUTH SUBNITRATE

pH at:—	Time in minutes after addition of 2g. bismuth subnitrate				
	0–20	20–40	40–60	60–80	80–100
0 secs. ..	1·42	—	—	—	—
30 secs. ..	1·48	1·51	1·45	1·44	1·46
5 mins. ..	1·5	1·52	1·45	1·45	1·45
10 mins. ..	1·52	1·45	1·44	1·45	1·44
15 mins. ..	1·52	1·45	1·44	1·46	1·43
20 mins. ..	1·52	1·45	1·45	1·46	1·43

made by comparing the amount of elemental bismuth which is required to neutralise 100 ml. 0·05N HCl viz.: In the carbonate 1·10 g., subnitrate 6·05 g., aluminate powder 0·139 g., aluminate cream 0·367 g.

The marked superiority of bismuth aluminate, especially the powder allows efficient, yet economical bismuth therapy.

Results from Our Own (MCP) Method

Results are recorded in the Tables III–V.

The results for bismuth subnitrate 2·0 g. are given in full to show the method of calculation of antipeptic activity. Tables for other tests show only the final results.

At the beginning of the experiment, the precipitation time of the artificial gastric juice used is determined. This time (Table IV) (1) in the

TABLE IV

ANTIPEPTIC PROPERTIES OF 2 G. OF BISMUTH SUBNITRATE

Volume at start 150 ml.	Time in minutes				
	0–20	20–40	40–60	60–80	80–100
(1) Precipitation time at time zero (seconds)	61	86	71	75	66
(2) Pepsin concentration mg. per cent at time zero ..	150	100	123	118	135
(3) Pepsin present in 150 ml. at time zero	225	150	184	177	203
(4) Pepsin added in period (mg.)	45	45	45	45	45
(5) Total pepsin at start (mg.)	270	195	229	222	248
(6) Precipitation time at the end of period (seconds) ..	86	71	75	66	63
(7) Pepsin concentration at the end of period (mg.per cent)	100	123	118	135	140
(8) Total pepsin at the end of period (mg.)	180	221	212	243	252
(9) Pepsin inactivated in the period (mg.)	90	0	17	0	0
(10) Pepsin re-activated in the period (mg.)	0	26	0	21	4
(11) Pepsin discarded at the end of the period (mg.) ..	30	37	35	40	—

Total pepsin used mg. = 450
Total pepsin inactivated mg. = 56
Total pepsin discarded mg. = 142
Total pepsin at the end mg. = 252

56 mg. pepsin inhibited by 2 g. bismuth subnitrate = 1·46 g. Bi., i.e. 38 mg. pepsin per 1 g. Bi.

} Pepsin inactivation in relation to amount of preparation used.

0–20 minute period) corresponds to 150 mg. pepsin per 100 ml. and provides a standard. Since the initial volume in each period is 150 ml., the pepsin present therein (3) is $1\frac{1}{2} \times$ the concentration (2). In each period 30 ml. of fresh artificial gastric juice is added and this contains 45 mg. of pepsin (4). The total amount of pepsin at the start (5) is

obtained by the addition (3) plus (4). The precipitation time at the end of each period (6) is converted into pepsin concentration (7). Since the volume at the end is 180 ml. the total pepsin at the end (8) equal $\frac{9}{5}$ × concentration. Pepsin inactivated or re-activated (9) and (10) is represented by the difference in total pepsin at the start and end of the period. 30 ml. of the mixture is discarded and this contains $\frac{3}{10}$ of pepsin concentration (mg. per cent) at the end (11).

The precipitation time of the pepsin concentration at the beginning of the second and subsequent periods is the same as at the end of the preceding period.

TABLE V

ANTACID AND ANTIPEPTIC PROPERTIES OF SUBSTANCES TESTED BY M.C.P. METHOD

Compound and its weight	pH at minutes						Total pepsin used = 450 mg. Pepsin inactivated: mg.	mg. pepsin inactivated per g. of element
	0	20	40	60	80	100		
Bismuth subnitrate .. .. 5 g.	1·4	1·54	1·56	1·58	1·55	1·55	74	20
Bismuth carbonate .. .. 2 g.	1·48	1·56	1·60	1·60	1·60	1·58	158	98
Bismuth carbonate .. .. 5 g.	1·51	1·53	1·55	1·55	1·55	1·53	377	94
Bismuth aluminate cream 11·0 ml. (0·7 g. Bi)	1·49	3·55	3·20	2·95	2·75	2·54	450	642+
Bismuth aluminate powder .. 2 g. (0·7 g. Bi)	1·50	3·77	3·55	3·35	3·10	2·70	450	642+
Aluminium hydroxide powder 3 g.	1·4	1·54	1·41	1·43	—	1·43	17	16·3
Aluminium hydroxide Gel BPC 5·4 g. (1 teaspoonful)	1·49	3·18	2·94	2·70	2·50	2·22	207	38
Aluminium hydroxide Gel BPC 10 g. (2 teaspoonsful)	1·49	3·88	3·75	3·55	3·42	3·22	378	37·8
Magnesium carbonate powder 0·1 g.	1·47	1·75	1·67	1·68	1·63	1·59	56	1900
Magnesium carbonate .. 1 g.	1·43	7·34	7·30	7·22	7·04	6·88	450	
Magnesium carbonate .. 2 g.	1·47	7·52	7·46	7·42	7·38	7·28	450	
Magnesium trisilicate powder 0·7 g.	1·44	1·82	1·98	1·78	1·62	1·63	47	361
Magnesium trisilicate .. 2 g.	1·41	2·78	3·00	2·78	2·55	2·28	272	715
Magnesium oxide powder .. 0·1 g.	1·5	1·90	1·78	1·73	1·69	1·65	10	166
Magnesium oxide .. .. 0·5 g.	1·4	9·50	9·30	9·20	8·88	8·70	450	
Sodium bicarbonate .. 1 g.	1·4	6·38	6·24	6·04	5·4	5·2	313	
Calcium carbonate powder 1 g.	1·49	5·53	5·42	5·37	5·18	4·88	18	
Calcium carbonate powder 3 g.	1·42	5·66	5·62	5·60	5·53	5·50	40	
Aluminium glycinate .. 2 g.	1·51	3·30	3·31	3·20	3·04	2·92	360	900
Aluminium glycinate .. 0·9 g.	1·58	2·80	2·72	2·58	2·41	2·30	211	1171

DISCUSSION

Within the conditions of the test all three bismuth salts have antipeptic power. In terms of bismuth content, bismuth aluminate is the most efficient.

It is difficult to explain why 2 g. of bismuth subnitrate should inactivate almost twice as much pepsin as 5 g. when the results are expressed in terms of mg. of pepsin inactivated per g. of bismuth. The experiments have been repeated on three occasions with similar results. With bismuth carbonate the indices for 2 doses (98 and 94) are sufficiently close to be recorded as the same. It is not possible to state the absolute antipeptic index for bismuth aluminate, since with both powder and cream, the quantitity used inactivated all the available pepsin. Subsequent tests have shown that there may be slight variation, in the antipeptic power, but this never falls below 600 mg. pepsin per g. bismuth.

The antacid results confirm opinion already expressed[1,2,10], i.e. that bismuth carbonate and subnitrate are poor antacids. Conversely bismuth aluminate powder by Brindle's method neutralises 70–88 per cent of acid in 20 minutes without an abnormal increase in pH and in therapeutic doses (Table V) using our own method maintains the pH of the stomach

TABLE VI

ANTACID AND ANTIPEPTIC INDEX OF SOME COMMONLY EMPLOYED ANTACIDS

		Antacid index*	Per cent pepsin inactivated	
Group 1				
Bismuth aluminate powder	2·0 g.	100	100	Good antacid and anti-peptic properties
Bismuth aluminate cream	11·0 ml.	100	100	
Aluminium glycinate	2·0 g.	100	80	
Aluminium hydroxide gel B.P.C.	10 g.	95	84	
Group 2				
Magnesium trisilicate	2·0 g.	95	60	Require potentiation of antipeptic effect
Aluminium glycinate	0·9 g.	100	47	
Aluminium hydroxide gel B.P.C.	5·4 g.	95	46	
Group 3				
Bismuth carbonate	5·0 g.	–	84	Requires potentiation of antacid effect
Group 4				
Bismuth carbonate	2·0 g.	–	35	Require potentiation of antacid and antipeptic effects
Bismuth subnitrate	2·0 g.	–	12	
Bismuth subnitrate	5·0 g.	–	16	
Magnesium carbonate	0·1 g.	–	12	
Magnesium trisilicate	0·7 g.	–	9	
Magnesium oxide	0·1 g.	15	2	
Aluminium hydroxide powder	3·0 g.	–	4	
Group 5				
Magnesium carbonate	1·0 g.	+	100	Unsatisfactory
Magnesium carbonate	2·0 g.	+	100	
Magnesium oxide	0·5 g.	+	100	
Calcium carbonate	1·0 g.	+	4	
Calcium carbonate	3·0 g.	+	9	
Sodium bicarbonate	1·0 g.	+	70	

* No. of minutes for which the acidity was within the range pH 2—4 (+ indicates pH above 4. – indicates pH below 2.)

within the desirable range of 2 to 4 throughout the 100 minute period of the test, whereas this was not so for the other bismuth salts. On the basis of this assessment bismuth aluminate is the most desirable of the three salts.

In comparing bismuth aluminate with the other substances tested, difficulties arise in that in some instances the increase of pH was of itself capable of inactivating pepsin. Thus magnesium oxide, 0·1 g. inactivates pepsin at the rate of 166 mg./g. of elemental magnesium whereas magnesium oxide 0·5 g. inactivates 1500 mg. of pepsin per g. of elemental magnesium. Under the conditions of these experiments pepsin became inactive at pH 5–6. With the small quantity of magnesium oxide the pH never rose above 2·12, and this could not account for pepsin inhibition. With the larger quantity, the pH was above 8·7 throughout.

The merits of each dose of each substance may be compared by giving them an antacid index and considering this together with the pepsin inactivated. The antacid index is defined here as the number of minutes for which the acidity was within the range pH 2–4. The pepsin inactivated is expressed as a percentage of the total pepsin used in the test.

Group 1

Substances in this group fulfil the *in vitro* requirements and therefore merit consideration as therapeutic agents. They can be relied upon to exert a desirable antacid action and at the same time inactivate pepsin. Differences of pH in the range pH 2·5–3·5 represent only a small percentage of acid and are not therefore important. Conversely differences in the pepsin inactivated as recorded in Table VI are of greater magnitude.

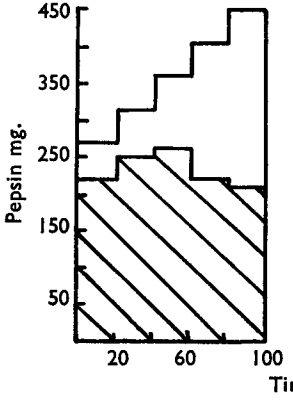

FIG. 3. Antipeptic action of 5·4 g. aluminium hydroxide gel BPC. See Table II. Note that after 60 minutes pepsin starts to be washed out and reactivated.

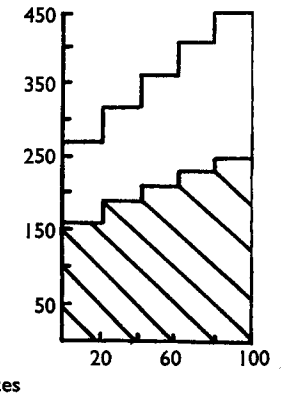

FIG. 4. Antipeptic action of a sub-minimal dose of bismuth aluminate powder. Note that although inactivation is incomplete there is no reactivation.

Unshaded area: active pepsin; shaded area: inactivated pepsin.

(1 per cent = 4·5 mg. pepsin.) Thus bismuth aluminate is a significantly better antipeptic than aluminium glycinate or aluminium hydroxide in the therapeutic doses tested. However, the antipeptic properties of aluminium glycinate would be improved by increased dosage, which would be unlikely to adversely effect the antacid index. Larger doses of glycine may be uneconomical and may give rise to undesirable side reactions such as gastro-intestinal hurry. A consideration of Table V and that of Figure 3 reveals that with aluminium hydroxide, while in the earlier 20 minute periods of the test, the pepsin inactivation is effective this power diminishes as the test proceeds, the pepsin being washed out of its combination with aluminium hydroxide. This phenomon is due to pepsin being adsorbed by aluminium hydroxide, but released again upon the addition of more HCl and the subsequent formation of soluble aluminium chloride.

Figure 4 expresses the results of a test with a subminimal dose of bismuth aluminate. Although the inactivation of pepsin is incomplete there is no evidence of pepsin being re-activated during the test.

Group 2

These substances are deficient in antipeptic effect. With aluminium glycinate this can be made good by increasing the dosage which also brings some improvement with aluminium hydroxide gel.

Group 3

Bismuth carbonate alone is an ineffective antacid and its chief use might well be to potentiate the effect of magnesium trisilicate, or some of the non-bismuth salts in Group 4.

Group 4

The preparations in this group are deficient in both antacid and antipeptic properties. With bismuth salts, the deficiency cannot be made good by increasing the dosage. Only in 3 times the B.N.F. dose is magnesium trisilcate a satisfactory antacid, and even then the antipeptic effect is poor. From Table V it can be calculated that to inactivate 450 mg. pepsin approximately 4 g. of MgO would be required, this would give far too great an antacid effect and thus this substance can only be of value when used in a mixture.

The dosage of aluminium hydroxide powder used (3 g.) is far in excess of the standard therapeutic dose, and yet both antacid and antipeptic effects are insignificant. The difference from the aluminium hydroxide gel is striking and emphasises the dangers inherent in assuming that such a salt in the dry state will have similar properties hydrated.

Group 5

All these substances gave an abnormally high gastric pH and must be considered unsuitable for therapeutic use.

Preparations administered in peptic ulcer may possess properties other than those discussed. For example, coating and sedative properties have not been considered. Furthermore, the final assessment of any therapetutic agent rests with its clinical appraisal. It is a necessary preliminary to clinical trial that the pharmacological properties of a compound should be assessed on a comparative basis. The scheme outlined here provides a method of doing this, and is considered preferable to older methods in that it includes inactivation of pepsin as well as acid neutralisation.

Acknowledgements. I wish to thank Mrs. Margaret O'Keeffe and other members of the Laboratory Staff who have assisted in this work. Also I should like to acknowledge the much needed and appreciated advice given by Dr. A. E. Davies, Ph.D., Chief Chemist, Mining & Chemical Products Limited.

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