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ANTIPYRETIC AND ANALGESIC PROPERTIES OF TWO HYDROXY*ISO*PHTHALIC ACIDS

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4-Hydroxy*iso*phthalic acid (4-HIPA) was first described by Barth (1872). Idris Jones and his co-workers (Reports of Chemistry Research Board, 1952, 1953) identified 4-HIPA as the main constituent of the "brown dust" residues from the sublimation of salicylic acid during manufacture. They suggested that 4-HIPA should be examined for similar pharmacological properties to salicylic acid and they provided material for this purpose. Pharmacological examination showed that this compound indeed possessed potent antipyretic and mild analgesic properties, which led us to examine also 2-hydroxy*iso*phthalic acid (2-HIPA), another by-product of the Kolbe-Schmitt process for the manufacture of salicylic acid. The results have been briefly reported (Chesher, Collier, Robinson, Taylor, Hunt, Idris Jones, and Lindsey, 1955). The present paper describes in detail the pharmacological work on both compounds.

METHODS

Antipyretic Tests.—Himalayan rabbits of either sex, weighing between 2.0 and 3.5 kg., which had been deprived of food and kept in an even laboratory temperature overnight, were placed in neck stocks in individual boxes. Rectal thermocouples were inserted to a depth of about 7 cm. Galvanometer readings were taken half-hourly to a total of 12 readings over a period of 5½ hr. After the third reading, each rabbit received intravenously per kg. 1 ml. saline containing 0.5 μg. of pyrogen prepared from *Proteus vulgaris* in the same way as fraction F68 of Miles (1951). After the fifth reading, suspensions of 4-HIPA, 2-HIPA, or aspirin were injected intraperitoneally, except in the control group. In order to reduce the readings in all rabbits to a comparable basis, each was expressed as the difference in °C. from the mean of the first three readings in the same animal.

Analgesic Tests.—Young Wistar rats of either sex within the weight-range 50–200 g. were used. Experiments reported below indicated that the same animal might safely be used on up to five occasions not more than twice weekly. In each experiment rats of about equal weight were distributed in dosage groups of ten or more and one group received a control solution, either of gum acacia-saline or of 5% sodium dihydrogen phosphate adjusted with N-HCl to the same *p*H as 4-HIPA suspensions (approximately 2.9). By means of a modified analgesiometer of Green, Young, and Godfrey (1951), in which the horizontal syringe was propelled by hand instead of by motor, we determined the pressure, applied to the tip of the rat's tail, required to elicit a squeak. This is called the squeak-threshold. An analgesic response was taken as a raising of the squeak-threshold to at least twice the geometric mean of the control group.

Local Anaesthetic Test.—Aqueous solutions of drugs were administered intradermally to guinea-pigs and the proportions of responses to needle pricks at the site of injection observed, according to the method of Somers and Edge (1947).

Toxicity Tests.—Albino male mice weighing 18–24 g. and male Wistar rats of 50–120 g. were used. In acute tests deaths were counted after seven days. In chronic tests drugs were mixed in a dry powdered diet (M.R.C. No. 41) and animals were weighed weekly. Blood haemoglobin, red cells, and white cells were estimated by conventional methods and prothrombin in plasma, diluted to 12.5% in saline, by the method of Quick, Stanley-Brown, and Bancroft (1935).

Administration of Drugs.—4-HIPA, 2-HIPA, aspirin, salicylic acid, salicylamide, and phenacetin were administered in suspension in 5% gum-acacia in 0.9% saline. Calcium 4-hydroxy*iso*phthalate, calcium aspirin, procaine hydrochloride, codeine, codeine phosphate, morphine hydrochloride, pethidine hydrochloride, nalorphine hydrobromide, pentobarbitone sodium, and thiopentone sodium were given in aqueous solutions and methylpentynol in saline. When salts were used weights of drugs are expressed as the active acid or base.

RESULTS

Antipyretic Experiments in Rabbits

The antipyretic activity of 4-HIPA is shown in the experiment illustrated in Fig. 1. Twenty

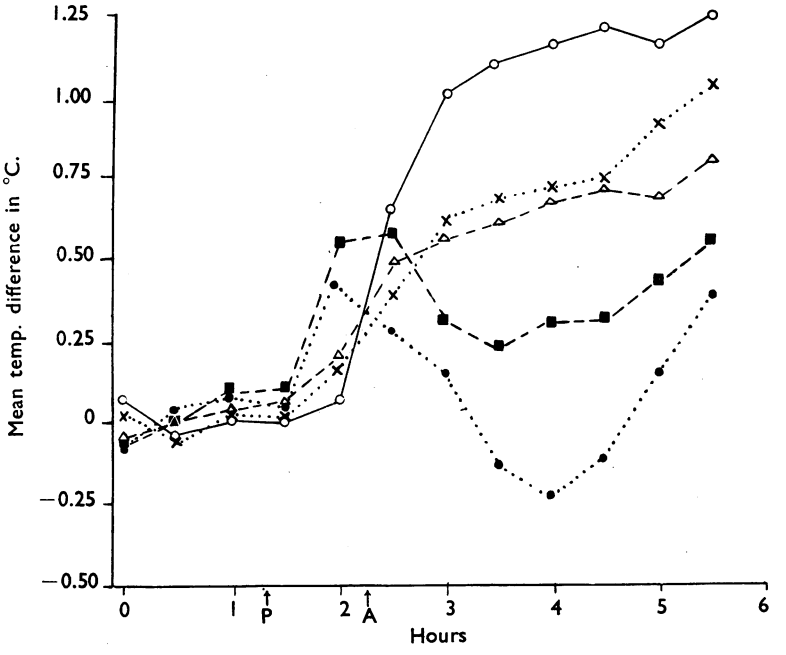

FIG. 1.—Antipyretic effects of 4-HIPA and aspirin on rabbits treated with *Proteus* pyrogen. Pooled results of a twin cross-over test in 20 rabbits. At P, 0.5 μg. pyrogen i.v.; at A, antipyretic injected i.p. O–O=pyrogen alone; ×···×=pyrogen+4-HIPA 22.2 mg./kg.; Δ–Δ=pyrogen+aspirin 22.2 mg./kg.; ■–■=pyrogen+aspirin 66.7 mg./kg.; ●··●=pyrogen+4-HIPA 66.7 mg./kg.

rabbits received pyrogen; 2 groups of 4 then received 4-HIPA or aspirin, either 66.7 or 22.2 mg./kg.; the remaining 4 animals being controls. Three days later the rabbits were again treated, except for one moribund animal which had received the higher dose of aspirin. The same rabbits were used as controls on both occasions, their mean maximum temperature rises being 1.26° C. on the first day and 1.34° C. on the second. On the second day of test the group that had received the higher dose of aspirin now received the higher dose of 4-HIPA, and so on.

The corresponding temperature differences obtained with the same dose of drug were pooled and their means plotted against time (Fig. 1). The temperature in all groups began to rise soon after the administration of pyrogen. In the control group, the temperature reached an approximate plateau within 2 hr.; in rabbits receiving 4-HIPA or aspirin as well as pyrogen, the rise was wholly or partly suppressed and sometimes replaced by a fall.

The antipyretic response of each rabbit was derived from its lowest temperature between 2 and 4 hr. after giving the pyrogen. These responses in the various dosage groups were compared by means of *t* tests. At the 66.7 mg./kg. dose level the response to 4-HIPA was not quite significantly greater than the response to aspirin ($P=0.1$–0.05); at the 22.2 mg./kg. dose level the difference in mean responses to the two drugs was negligible. In further comparative tests, 24 rabbits received pyrogen; 8 of these then received 66.7 mg./kg. of 4-HIPA, and 8 a similar dose of aspirin. The mean lowest temperature difference after 4-HIPA was 0.14° C. and after aspirin 0.11° C., while the mean maximum rise in the controls was 1.46° C. We conclude that 4-HIPA and aspirin possess a similar degree of antipyretic activity.

In a further experiment, 12 rabbits were treated with pyrogen. Four of these then received 200 mg./kg. 2-HIPA and another four an equal dose of 4-HIPA. Both groups showed a comparable antipyretic response.

Analgesic Experiments in Rats

Single Doses.—Single large but not lethal doses of 4-HIPA, given intraperitoneally to rats, raised their squeak-thresholds significantly above those of control animals receiving buffer solution at the same *p*H. The analgesia was unaccompanied by loss of righting reflex, drowsiness or other visible side-effects. Although we failed to detect anal-

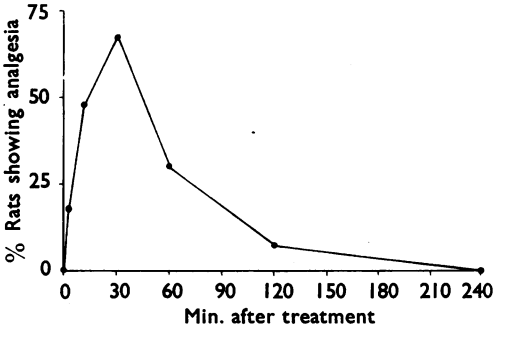

FIG. 2.—Time-course of analgesia in rats after intraperitoneal injection of 450 mg./kg. 4-HIPA.

gesia in most rats when 4-HIPA was given entirely by the oral or subcutaneous routes, the proportion of analgesic responses could be increased by giving a large oral dose, together with a just effective intraperitoneal dose.

In experiments on the time-course of analgesia, 450 mg./kg. 4-HIPA was administered intraperitoneally to rats, and at various times afterwards groups of 20 were tested by analgesiometer. At 3 min. after treatment the squeak-threshold had risen in some animals; at 30 min. the proportion of rats showing analgesia reached its peak, and by 4 hr. the response had disappeared (Fig. 2). In animals destroyed 6 hr. after treatment no traces of 4-HIPA could be detected in the peritoneal cavity. In further analgesic experiments the squeak-threshold was measured at 30 min. after treatment.

TABLE I

POTENCIES AND TOXICITIES OF SOME ANALGESICS IN RATS

Codeine administered subcutaneously in aqueous solution, remaining drugs intraperitoneally in gum acacia-saline

Drug	Potency		Toxicity	
	No. of Rats	ED50 (Fiducial Limits)	No. of Rats	LD50 (Fiducial Limits)
4-HIPA ..	968	315 (279 to 354)*	79	1,071 (968 to 1,185)*
2-HIPA ..	110	356 (286 ,, 443)*	20	925 (892 ,, 957)†
Codeine ..	98	11·5 (9·3 ,, 13·7)†	35	372 (297 ,, 447)†
Aspirin ...	—	—	76	541 (485 ,, 603)*
Salicylamide	60	175 (165 ,, 187)†	40	592 (573 ,, 610)†

* Computed. † Graphical estimate.

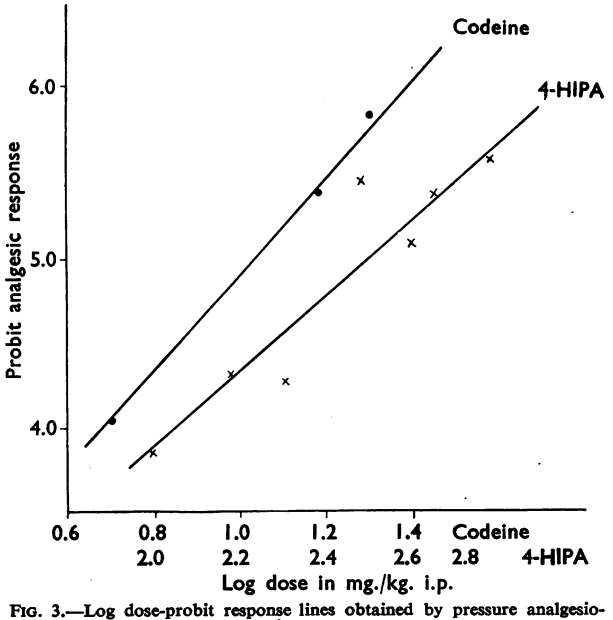

FIG. 3.—Log dose-probit response lines obtained by pressure analgesiometer in young rats.

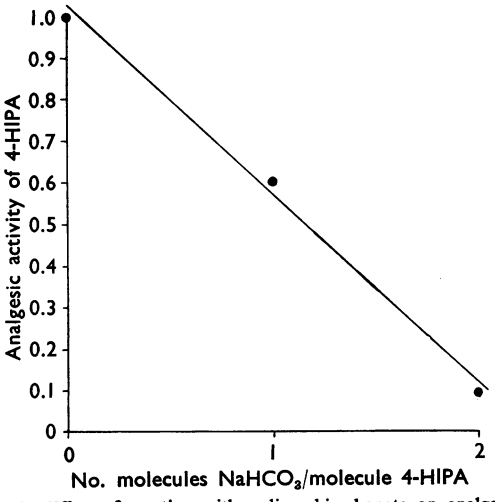

FIG. 4.—Effect of reaction with sodium bicarbonate on analgesic activity of 4-HIPA. Activity of 4-HIPA alone = 1.0.

In Table I are expressed the analgesic activities of 4-HIPA, 2-HIPA, codeine and salicylamide. The ED50 of salicylamide was identical with that found by Way, Takemori, Smith, Anderson, and Brodie (1953), who used a similar technique. 2-HIPA and codeine resembled 4-HIPA in lack of visible side-effects in the rat. When codeine and 4-HIPA were administered intraperitoneally, their probit-log dose lines were approximately parallel (Fig. 3) and we therefore compared directly their analgesic activities by this route. From experiments in 381 rats, 4-HIPA possessed 4.16% the potency of codeine (fiducial limits, 3.21 to 5.54). We did not compare 4-HIPA directly with salicylates, because aspirin and salicylic acid in just tolerated doses (300 and 400 mg./kg. respectively) failed to produce a significant analgesic response, and salicylamide caused sleepiness as well as analgesia, as described by Berger (1954).

4-HIPA is relatively insoluble in water (1 in 5,000 at 20° C.); but the product of mixing sodium bicarbonate and 4-HIPA in cold water in proportion of 2 molecules $NaHCO_3$ to 1 molecule 4-HIPA was more soluble. When such a solution, of *p*H 6.8, was injected intraperitoneally, its analgesic activity and toxicity were very low, the activity being about one-tenth that expected from the 4-HIPA added to the reaction mixture. By the intravenous route the solution was also of low activity.

In order to explore this effect still further, we examined the analgesic potency of an equimolecular mixture of $NaHCO_3$ and 4-HIPA in water, which formed a suspension of *p*H 4.4. Its analgesic activity was about 60% that expected from the 4-HIPA added to the reaction mixture. The results of these experiments are expressed in Fig. 4.

Repeated Administration.—Groups of 10 rats were dosed intraperitoneally twice weekly for 3 weeks with 450 or 150 mg./kg. 4-HIPA, with 15 or 5 mg./kg. codeine or with 10 ml./kg. saline. After each administration, rats were tested by analgesiometer. Over this period there was no significant change in the analgesic response to either drug. After the 4th dose, 1 rat receiving 450 mg./kg. 4-HIPA died.

During a period of 5 weeks, 8 intraperitoneal doses of 200 mg./kg. 4-HIPA or aspirin or of 10 ml./kg. phosphate buffer at *p*H 2.9 or saline were administered to groups of 10 rats. During these weeks, 1 rat receiving aspirin and 1 receiving saline died. At the end of the period, all survivors were treated intraperitoneally with 15 mg./kg. codeine and tested by analgesiometer. Four days later the experiment was repeated, using 450 mg./kg. 4-HIPA instead of codeine. Both control groups gave normal responses to both analgesics, but the drug-treated animals showed reduced responses. At the end of this experiment the animals were destroyed. Post-mortem observations are discussed below under the heading of subacute toxicity.

Local Anaesthetic Experiments

To examine the possibility that the calcium salt of 4-HIPA possessed local anaesthetic activity, we compared it with procaine hydrochloride and calcium aspirin, using a calcium chloride solution of equivalent calcium content as control. In these experiments, calcium 4-HIPA and calcium aspirin showed significant but very slight local anaesthetic activity, which was about 3% that of procaine.

5% solutions of the calcium salt of 4-HIPA and of calcium aspirin caused local necrosis after intradermal injection.

Toxicity

Acute.—The acute toxicities to rats of 4-HIPA, 2-HIPA and various other analgesics with which they were compared are summarized in Table I.

Subacute.—The rats, which had received 8 doses of 4-HIPA, aspirin or control solutions during 5 weeks in the experiment described above, were destroyed and examined *post mortem*. In all drug-treated animals, the livers were pale and swollen and the liver capsules and other parts of the peritoneum were thickened and adherent. There was also some blood-stained fluid in the peritoneal cavity. On histological examination of the livers, the only change detected was capsular hypertrophy.

Chronic.—In a preliminary chronic toxicity test, over a period of 14 weeks, 2 of 10 mice receiving 0.5% and 3 of 10 receiving 1% 4-HIPA in their diet died. In corresponding dosage groups receiving aspirin, there were 3 and 2 deaths respectively.

Rats were maintained for 26 weeks on diets containing 0.2 or 0.1% 4-HIPA or aspirin. All animals survived; but towards the end of this period one rat on 0.2% 4-HIPA lost weight and showed abdominal enlargement. Blood examinations (Table II) did not show any substantial difference between treated and control rats, although the white cell counts were low throughout. The differences in prothrombin time, however, between untreated rats and those receiving 0.2% 4-HIPA ($P=0.1$) or aspirin ($P=0.2$) approached significance. The growth curves of the untreated rats and those receiving the higher percentages of 4-HIPA or aspirin are shown in Fig. 5, from which it will be seen that aspirin, and to a lesser extent 4-HIPA, slightly depressed the rate of growth.

Histological examination of sections of brain, stomach, small and large intestine, liver, thyroid,

TABLE II

MEAN VALUES OBTAINED FROM BLOOD EXAMINATION OF RATS WHICH HAD RECEIVED 4-HIPA OR ASPIRIN IN THEIR DIET FOR 26 WEEKS

L=lymphocytes; M=monocytes; N=neutrophils; E=eosinophils; B=basophils

% Drug in Diet	% Haemoglobin	Erythrocytes per $mm^3 \times 10^6$	Leucocytes per $mm^3 \times 10^3$	Differential Counts %				Prothrombin Time in Sec.
				L and M	N	E	B	
0·2 4-HIPA	108	8·6	4·5	73·55	23·40	3·00	0·05	28·3
0·1 „	108	9·1	5·0	73·80	24·00	2·20	0·00	26·1
0·2 aspirin	106	8·6	4·4	74·60	22·25	3·15	0·00	28·2
0·1 „	113	8·7	5·0	81·90	15·15	2·90	0·05	27·1
Untreated controls ..	110	8·4	4·2	79·80	18·10	2·05	0·05	25·2

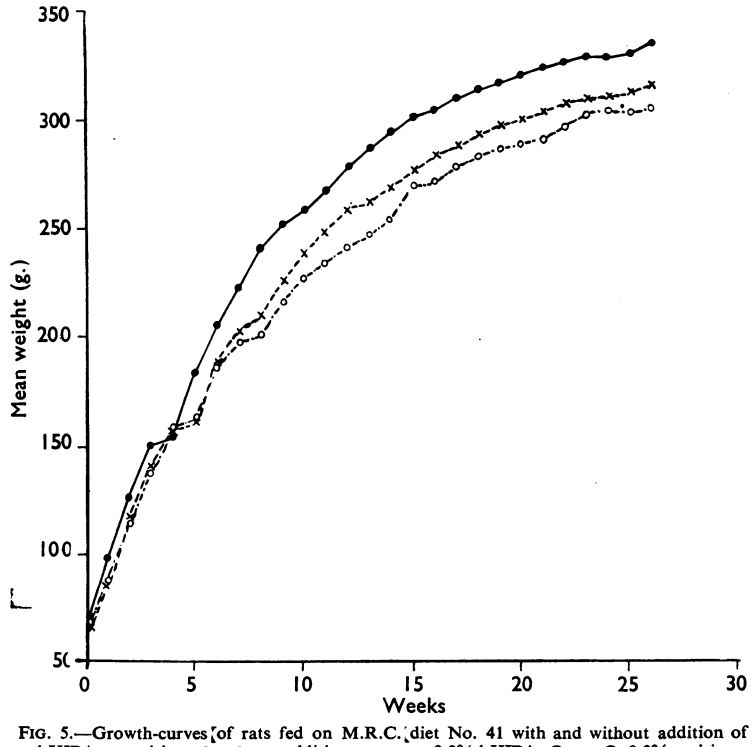

FIG. 5.—Growth-curves of rats fed on M.R.C. diet No. 41 with and without addition of 4-HIPA or aspirin. ●—●, no addition; ×--×, 0.2% 4-HIPA; O-.-O, 0.2% aspirin.

spleen, kidney, heart, lung, and testis of all rats in the chronic toxicity test showed no pathological effects attributable to 4-HIPA or aspirin. The rat mentioned above, which lost weight and showed abdominal enlargement, had a glandular hyperplasia resembling an adenocarcinoma in the large intestine. This was not attributed to the drug treatment.

Antagonism and Synergism

In view of the possible clinical use of 4-HIPA and because light might be thrown on its mode of action, we examined the effects of administering to rats a number of established drugs jointly with 4-HIPA.

Nalorphine. — Nalorphine was administered subcutaneously 20 min. before an intraperitoneal dose of 15 mg./kg. codeine or 450 mg./kg. 4-HIPA, the dose-ratio of nalorphine to each analgesic being 1 to 2. Nalorphine completely suppressed the analgesic effect of codeine; but it had no effect on that of 4-HIPA.

Other Analgesics. — Administering codeine subcutaneously and 4-HIPA intraperitoneally, we determined in the same experiment the analgesic potencies of the drugs given alone and together. The pooled results of three experiments are expressed graphically by the method described by Gaddum (1953) in Fig. 6(a), where each point was obtained with 40 rats. In this figure the ED50 isobol falls below the line of addition, indicating potentiation. Similar experiments in which we determined acute toxicity are expressed in Fig. 6(b), which indicates that toxicities were approximately additive.

Analgesic experiments of a similar type with 2-HIPA and codeine, in a total of 220 rats, also showed potentiation. In further experiments, morphine, pethidine, and phenacetin failed to potentiate, but showed an additive effect with 4-HIPA.

Hypnotics.—Unlike 4-HIPA, the hypnotic drugs studied caused the rat to lose its righting reflex; but doses ineffective in this way did not produce analgesia in our test. In experiments on synergism, we determined the effect of a constant dose

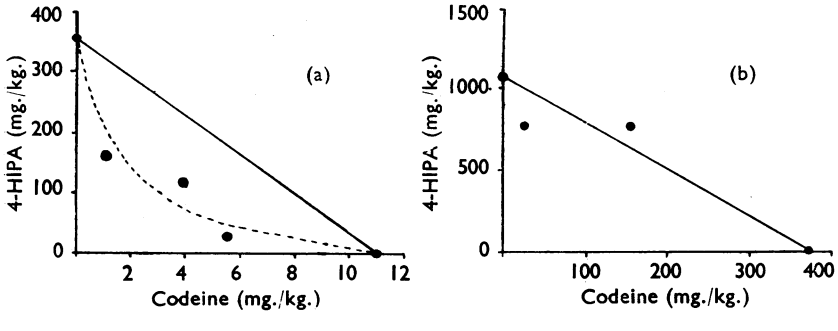

FIG. 6.—Synergism of 4-HIPA and codeine. 4-HIPA administered intraperitoneally, codeine subcutaneously, to young rats. (a) Analgesic ED50's; (b) LD50's.

of one drug on the potency of the other. Table III illustrates the enhancement of the analgesic activity of 4-HIPA by 100 mg./kg. methylpentynol and Table IV the enhancement of the hypnotic activity of methylpentynol by 450 mg./kg. 4-HIPA.

TABLE III

ENHANCEMENT OF ANALGESIC ACTIVITY OF 4-HIPA BY METHYLPENTYNOL

Both drugs were given intraperitoneally

Methylpentynol (mg./kg.)	4-HIPA (mg./kg.)	Proportion of Rats Showing Analgesia	ED50 (mg./kg.)
100	—	0/20	—
—	450	12/20	
—	150	7/20	363
100	150	14/20	
100	50	7/20	79

TABLE IV

ENHANCEMENT OF HYPNOTIC ACTIVITY OF METHYLPENTYNOL BY 4-HIPA

Both drugs were given intraperitoneally

Methylpentynol (mg./kg.)	4-HIPA (mg./kg.)	Proportion of Rats Losing Righting Reflex	ED50 (mg./kg.)
—	900	0/10	—
300	—	6/10	
225	—	2/10	282
200	—	0/10	
200	450	6/10	
150	450	4/10	174

In acute intraperitoneal toxicity tests, the LD50 of methylpentynol alone was 537 mg./kg. and that of methylpentynol in the presence of an equal dose of 4-HIPA was 555 mg./kg. These figures give no evidence of synergism in toxicity between the two drugs.

Experiments similar to those with methylpentynol were performed with thiopentone or pentobarbitone and 4-HIPA. 10 mg./kg. of either barbiturate alone had no analgesic action, but more than doubled the analgesic potency of 4-HIPA. Conversely, 450 mg./kg. 4-HIPA raised the hypnotic potency of thiopentone by 1.7 and of pentobarbitone by 1.8 times.

In toxicity tests, the LD50 of pentobarbitone alone was 86 mg./kg. and in the presence of 13 times its weight of 4-HIPA was 58 mg./kg. There thus appears to be slight synergism in toxicity between the barbiturate and 4-HIPA.

DISCUSSION

The failure of nalorphine to antagonize 4-HIPA strengthens the argument that this compound does not belong to the morphine type of analgesic. Since both 2- and 4-HIPA exhibit antipyretic properties, we may place them in the group of analgesic-antipyretics, of which aspirin is the most widely used. Absence of drowsiness in rats at analgesic doses, however, marks a difference of 2- and 4-HIPA from salicylamide and phenacetin.

In rats, the acute toxicity of 4-HIPA was less than half and the chronic toxicity no higher than that of aspirin. Aspirin failed to show analgesic action at the highest tolerated dose given (300 mg./kg.); on the other hand, 300 mg./kg. 4-HIPA produced analgesia in about half the animals treated and higher tolerated doses were effective in nearly all rats. The acute toxicity of 4-HIPA was rather lower than that of 2-HIPA. One of our colleagues, who was strongly hypersensitive to aspirin, did not show any reaction to 4-HIPA. These facts pointed to the clinical trial of 4-HIPA as an alternative to aspirin.

Although admixture with sodium bicarbonate lowered the *in vivo* activity of 4-HIPA, the analgesic effect of the free acid in the rat was clearly specific and not due to acidity, since buffer solutions at the same *p*H were used as controls. Moreover, related acids, such as *o*-, *m*-, and *p*-phthalic and 5-hydroxy*iso*phthalic, showed much less activity. It also seems unlikely that the low activity of the 4-HIPA–$NaHCO_3$ mixture was due to failure of absorption from the peritoneal cavity, since a similar mixture was of low activity intravenously. Robinson, Fehr, and Fitzgerald (1956) have found that the rate of urinary excretion of 4-HIPA after giving the sodium salt intraperitoneally is much higher than after the free acid. This suggests that the effect may result from an increased rate of clearance of the sodium salt.

After 8 intraperitoneal doses of 4-HIPA or aspirin, rats became insensitive to both codeine and 4-HIPA administered by the same route. This effect may be attributable to the peritoneal hypertrophy, which was seen *post mortem*.

Most rats receiving 4-HIPA by the oral route failed to show an analgesic response; but an oral dose enhanced the effect of a simultaneous intraperitoneal dose. This is consistent with the finding of Robinson *et al.* (1956) that rats absorbed 4-HIPA much less completely from the gut than from the peritoneal cavity.

The main biological actions of 2- and 4-HIPA were expected from their structural similarity to salicylic acid. It is therefore surprising that, although both 2- and 4-hydroxy*iso*phthalic acids have been known for over 80 years, these properties do not seem to have been reported previously. We found similar properties also, though to a lesser

degree, in the unsubstituted phthalic acids (Chesher *et al.*, 1955) and related compounds.*

Summary

1. In rabbits treated with *Proteus* pyrogen, 4-hydroxy*iso*phthalic acid (4-HIPA), given by the intraperitoneal route, exhibited antipyretic activity at least as great as that of aspirin. 2-Hydroxy-*iso*phthalic acid (2-HIPA) also showed antipyretic activity.

2. Using a tail-pressure method in rats, 2- and 4-HIPA, administered intraperitoneally, showed analgesic action unaccompanied by loss of righting reflex, drowsiness or other visible side-effect. 2- and 4-HIPA were of about equal potency, which was greater than that of aspirin, but less than those of salicylamide and codeine.

3. In acute experiments in rats, 2-HIPA was weight for weight less toxic than codeine, aspirin or salicylamide: 4-HIPA was less toxic than 2-HIPA.

4. The subacute and chronic toxicity of 4-HIPA in mice and rats was of the same order as that of aspirin. When 0.2% 4-HIPA was administered for 6 months in the diet to young rats, no toxic effects were seen except a very slight depression of growth-rate.

5. Nalorphine did not antagonize the analgesic activity of 4-HIPA.

6. Codeine potentiated the analgesic activities of 2- and 4-HIPA. Methylpentynol, pentobarbitone and thiopentone enhanced the analgesic activity of 4-HIPA, and 4-HIPA enhanced the hypnotic action of these three drugs.

**Note added in proof.* The analgesic activities of some related acids in comparison with 4-HIPA (=1.0) were: *o*-phthalic, 0.4; *m*-phthalic (*iso*phthalic), 0.4; *p*-phthalic (*tere*phthalic), 0.5; 5-hydroxy*iso*phthalic, 0.3; 4,5-dihydroxy*iso*-phthalic, 0.9; hydroxy*tere*phthalic, 0.7; 2,3-dihydroxy*tere*phthalic, 0.6; 2,5-dihydroxy*tere*-phthalic, 0.6; trimesic, 0.5; hydroxytrimesic, 0.5; 2,4,6-trihydroxybenzoic (phloroglucinol carboxylic), 0.5. Some of the compounds were kindly supplied by Drs. J. Idris Jones, N. R. Campbell, J. H. Hunt and E. P. Taylor.

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