The abnormal hemoglobin derivative developed is not methemoglobin but is sulphurhemoglobin. This compound is formed by the action of hydrogen sulphide on the blood, and as acetanilid contains no sulphur it cannot be the cause of the condition.

CONCLUSIONS.

1. The toxic dose of acetanilid is about 100 times the therapeutic dose.
2. The M. L. D. in general agrees for all laboratory animals and is around 1500 mg. per Kg. or 500 times the therapeutic dose.
3. Continuous ingestion of large amounts has no deleterious effects upon animals.
4. Acetanilid is not a circulatory depressant.
5. Twelve grains a day for 16 weeks has no deleterious effect upon human subjects.
6. In the hospital records of the United States, cases of poisoning and deaths from the drug are of insignificant number.
7. In the hands of the Medical Profession, the use of these analgesic drugs has largely been replaced by the use of intellectually depressant, or sleep-producing, drugs.
8. The cyanosis seen at times in connection with the use of these drugs is not dependent upon drug action, but upon an individual predisposition of the user.

EMERSON TOWER BUILDING,
BALTIMORE, MD.

THE CHEMICAL AND PHARMACOLOGICAL PROPERTIES OF CALCIUM ACETYLSALICYLATE.*[1]

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During the past year, we have had an opportunity to examine several powder and tablet preparations of calcium acetylsalicylate. Inasmuch as this substance is marketed abroad while little information concerning it is available in American literature, we thought it worth while to record our observations with respect to its chemical, physical and pharmacological properties.

The following preparations were available for testing:

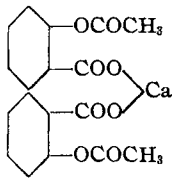
1. Kalmopyrin. Tablets and powder of calcium acetylsalicylate manufactured by the Chemical Works of Gideon Richter, Budapest.
2. Calcium Acetylsalicylate. Tablets and powder made for us by Arner and Company, Buffalo, New York.
3. Calcium Acetylsalicylate. Powder prepared in the laboratory.

* Aided by a grant from the Wodlinger fund.

[1] Scientific Section, A. PH. A., Madison meeting, 1933.

CHEMICAL COMPOSITION.

Calcium acetylsalicylate is a definite chemical compound $[C_6H_4O(CH_3CO)\text{-}COO]_2Ca$ with the following structural formula:



METHOD OF PREPARATION.

Several methods of preparation are employed, which consist for the most part of neutralizing solutions of acetylsalicylic acid with some calcium base (1, 2).

In the study of the chemical, physical and pharmacological properties of calcium acetylsalicylate, a comparison was made with U. S. P. preparations of acetylsalicylic acid obtained on the open market (Merck, Bayer). The following observations were made:

1. Solubility.

The solubilities of the various preparations were determined by the method of the U. S. P. X and are expressed in the following table (averages of four samples).

Preparation: Cc. of Solvent Required to Dissolve 1 Gm.

	Water.	Alcohol.	Ether.	Chloroform.	0.4% HCl.	Cottonseed Oil.	Dog's Gastric Juice.
Calcium acetylsalicylate (unhydrolyzed)	6	416	1428	1428	12.9	Not clearly soluble	Slowly soluble
Kalmopyrin	5[1]	400	435	666	12.7	Not clearly soluble	Slowly soluble
Acetylsalicylic acid (Merck)	300	5	10–15	17	..	Not clearly soluble	Slowly soluble

[1] Not clearly soluble in water.

2. Tests for Identity and Purity.

a. Reaction to Indicators.—Calcium acetylsalicylate (domestic) is neutral or faintly acid to litmus. It does not change color when phenolphthalein is added to an aqueous solution. Calcium acetylsalicylate (Kalmopyrin) is slightly acid to litmus.

b. Tests for Identity U. S. P. Method.—The *melting point* of calcium acetylsalicylate (domestic) is 100–105° C.; of calcium acetylsalicylate (foreign) is not definite (about 85° C.), while that of acetylsalicylic acid is 132° C.

Other tests for the identity of calcium acetylsalicylate correspond to those of acetylsalicylic acid U. S. P.

c. Tests for Purity U. S. P.

Solution.	T.S. of Lead Acetate.	T.S. of Silver Nitrate.	T.S. Mercuric Chloride.	T.S. of Ammonium Oxalate.	Color with Ferric Chloride T.S.
1. Calcium acetylsalicylate (unhydrolyzed)	No ppt.	Gelatinous ppt.	No ppt.	White ppt. (Ca)	Muddy brown but no violet color

2. Calcium acetylsalicylate (Kalmopyrin)	No ppt.	Gelatinous ppt.	No ppt.	White ppt. (Ca)	Brown violet
3. Acetylsalicylic acid (Merck)	No ppt.	Gelatinous ppt.	No ppt.	No ppt.	Faint violet

The U. S. P. tests for organic impurities were negative in all three salts.

3. Stability.

In order to determine the stability of the calcium acetylsalicylate preparations, they were examined upon receipt for odor, appearance and the presence of free salicylic acid. This examination was repeated at monthly intervals for five months, with the results appearing in the following table:

Preparation.		At Time of Receipt.	30 Days.	60 Days.	90 Days.	120 Days.	150 Days.
Calcium acetylsalicylate (domestic)	Odor	Earthy	No change	No change	No change	Slightly acetic	Acetic
	Appearance	Fine powder	No change	No change	No change	Slightly acetic	Slight change
	Free salicylic acid[1]	Negative	Negative	Negative	Negative	Trace	Definite
Calcium acetylsalicylate (Kalmopyrin)	Odor	Slightly acetic	Acetic	Acetic	Strongly acetic	Strongly acetic	Strongly acetic
	Appearance	Fine powder	Same	Fine crystalline deposit			
	Free salicylic acid	Trace	Definite to ——————————→				Marked
Acetylsalicylic acid U. S. P.	Odor	Earthy	No change throughout				
	Appearance	Fine powder	No change throughout				
	Free salicylic acid	Slight trace	No increase on standing				

[1] Attention should be called to the fact that the ordinary color reaction between free salicylic acid and ferric chloride is interfered with by the presence of calcium. The A. O. A. C. method of testing the ether extract should be employed.

The essential physical and chemical properties of calcium acetylsalicylate may be summarized as follows: It is freely soluble in water and very slightly soluble in ether, alcohol and chloroform while the reverse is true for acetylsalicylic acid. The differences between calcium acetylsalicylate and acetylsalicylic acid with respect to the various tests for identity and purity are dependent on the calcium (and chloride?) content. Upon first examination, the foreign preparation of calcium acetylsalicylate (Kalmopyrin) contained relatively large amounts of free salicylic acid in contrast with the domestic preparation and with acetylsalicylic acid. However, it was found that the domestic preparation was not permanently stable and as time went on, more and more free salicylic acid appeared. This instability was thought at first to be due to the methods of manufacture and calcium acetylsalicylate was prepared in the laboratory by a variety of methods. None, however, was successful in yielding a stable product. It was uniformly observed that the tablet preparations of calcium acetylsalicylate were more rapidly hydrolyzed than the powder presumably owing to the moisture necessary in tablet manufacture. Tablets kept in cardboard boxes preserved a good appearance

although containing considerable free salicylic acid, while those kept in tightly stoppered containers became moist, discolored, and exhibited crystals of salicylic acid on the surface.

PHARMACOLOGICAL STUDIES.

Prior to the time that it became apparent that calcium acetylsalicylate was unstable, an investigation of the pharmacological actions of this preparation was undertaken particularly with reference to a comparison with similar actions of acetylsalicylic acid. The essential findings are indicated in the following sections:

1. Absorption and Excretion.

Five healthy male adults ranging in age from 20 to 25 years were given 10-grain doses of calcium acetylsalicylate or acetylsalicylic acid with a small amount of water about two hours after the noon meal. The urine was examined at intervals and the time of the first appearance as well as the duration of excretion of salicylic acid (or salicyluric acid) in the urine determined. In ten experiments with calcium acetylsalicylate, salicylic acid appeared in the urine in 20, 40, 30, 30, 20, 30, 60, 30, 30 and 35 minutes, respectively, with an average of 32 minutes. In five experiments with acetylsalicylic acid, the results were 120, 60, 60, 45 and 120 minutes, respectively, with an average of 81 minutes. The average duration of excretion of salicylic acid in the urine after calcium acetylsalicylate administration was 33 hours, while with the acetylsalicylic acid it was 26 hours.

From these results, it appears that calcium acetylsalicylate is somewhat more rapidly absorbed but more slowly eliminated than acetylsalicylic acid.

2. Gastric Irritation.

An important consideration in salicylate medication is the irritant effect of such preparations upon the gastro-intestinal tract which may contribute to the nausea and vomiting which salicylates produce by central action after their absorption. Consequently it was considered advisable to compare the action of the calcium acetylsalicylate compounds with acetylsalicylic acid in this regard. This was done by intensive administration of the compounds to healthy dogs and noting the incidence of vomiting. A single dose of 1 Gm. per Kg. of either acetylsalicylic acid, calcium acetylsalicylate (Kalmopyrin) or calcium acetylsalicylate (unhydrolyzed) regularly induced vomiting in all dogs. Similarly vomiting regularly resulted in all dogs after two doses of 0.33 Gm. per Kg. each, repeated in 90 minutes. With a dose of 0.166 Gm. per Kg. repeated at intervals of 30 minutes, vomiting occurred with Kalmopyrin after the second dose, after the fourth dose of acetylsalicylic acid and after the fifth dose of the (unhydrolyzed) calcium acetylsalicylate. The foreign preparation of calcium acetylsalicylate (Kalmopyrin) which contained definite amounts of free salicylic acid is obviously more irritating than the other two. There is little or no difference in this regard between acetylsalicylic acid and the unhydrolyzed calcium acetylsalicylate. That the vomiting induced by these large doses administered in frequent intervals is reflex in character and due to gastric irritation is indicated both by the rapidity with which the emesis results after the administration and also by the fact that the emesis was produced with significantly less amounts of salicylate than can be tolerated

if the administration is such as to minimize the opportunities of irritation to the stomach. If, however, a dose is selected which is below the irritating dose and given at such a time interval that the administration is in excess of excretion, vomiting will then eventually occur, presumably due to the central action of the salicylates upon the vomiting center. That the salicylates may induce vomiting both by local gastric irritation and central action is also indicated at times by the effect of the single administration of very large doses. Immediate vomiting (due to local irritation) results. Then after three or four hours vomiting recurs apparently due to the central action of the salicylates which were not expelled by the vomiting.

3. Toxic Effect on the Kidneys.

To determine the effect of these compounds upon the kidney, they were administered to healthy dogs in various dosages and time intervals and the incidence of albuminuria noted. Acetylsalicylic acid and the unhydrolyzed calcium acetylsalicylate in doses of 0.100 Gm. per Kg. per hour for five doses did not produce an albuminuria. Acetylsalicylic acid in doses of 0.166 Gm. per Kg. four times daily induced an albuminuria when the total dosage given had reached 0.800 Gm. per Kg. Unhydrolyzed calcium acetylsalicylate in doses of 0.166 Gm. per Kg. four times daily did not induce an albuminuria and the dog was sacrificed when the total dosage given had reached 6.0 Gm. per Kg. Although the number of animals employed is small, there is an indication of a marked difference between the compounds in their effect upon the kidney. Kalmopyrin is considerably more irritating than the other two, apparently due to the free salicylic acid present. On the other hand, the unhydrolyzed calcium acetylsalicylate appears to be definitely less irritating than acetylsalicylic acid.

4. Toxicity.

Another important consideration in the intensive medication with the salicylates is the development of the so-called secondary signs of toxicity. In an effort to determine if any differences in the onset of the secondary symptoms of toxicity could be demonstrated, the compounds were administered in varying dosage levels to dogs. The onset of toxicity was determined by one or more of the following symptoms, convulsions, marked vertigo and staggering gait, marked asthenia or apparent disturbances in vision and hearing. At a dosage of 0.33 Gm. per Kg. repeated after 4 hours, dogs regularly vomited after the second dose with all compounds as indicated previously and it was not possible to continue administration at this dosage level. In the case of Kalmopyrin there was some evidence of toxic symptoms after the second dose.

At a dosage level of 0.165 Gm. per Kg. repeated at intervals of three times daily toxic symptoms developed with Kalmopyrin after the second dose, with acetylsalicylic acid after the twenty-eighth dose and with unhydrolyzed calcium acetylsalicylate after the thirty-seventh dose. At a dosage level of 0.100 Gm. per Kg. repeated at hourly intervals, toxic symptoms developed with Kalmopyrin after the second dose, with acetylsalicylic acid after the fourth dose and with calcium acetylsalicylate after the fourteenth dose. The dog receiving the acetylsalicylic acid died after the fourteenth dose. The one receiving the calcium acetyl-

salicylate was carried for several days longer and the administration then discontinued.

It is readily apparent from these results that Kalmopyrin is definitely more toxic than either of the other two compounds. There is likewise some indication that the unhydrolyzed calcium acetylsalicylate is less toxic than acetylsalicylic acid. The difference in toxicity appears to be greater than the difference in salicylate content of these compounds. From these results, it is not possible to state whether this apparent difference is due to a protective action of the calcium or to an alteration in the absorption and excretion rate which would vary the time required for the cumulative effect of these compounds to reach a toxic level in the blood or tissues.

It is difficult to determine the acutely fatal dose of such compounds as acetyl salicylic acid—as large doses given by mouth are promptly vomited and the compounds are too insoluble to be given readily by vein. For calcium acetylsalicylate, the intravenous fatal dose can be determined readily as it is sufficiently soluble. For calcium acetylsalicylate, the intravenous fatal dose in dogs was found to be between 0.60 and 0.75 Gm. per Kg.

SUMMARY.

From this study, the following remarks seem warranted. The unhydrolyzed calcium acetylsalicylate has certain advantages over acetylsalicylic acid in that it is more soluble in water, more readily absorbed, has less tendency to produce an albuminuria and is apparently less toxic as judged by the development of the so-called secondary signs of toxicity. It is apparently unstable, however, and as hydrolysis continues with the liberation of free salicylic acid, etc., it becomes more irritating to the gastric mucosa, more toxic and more likely to induce an albuminuria. This is illustrated by the results with Kalmopyrin in which such hydrolysis was quite marked.

REFERENCES.

(1) Coplans Myer, Patentschrift Nr. 534785, October 1, 1931.
(2) J. Altwegg, U. S. Patent Office, No. 1,431,863.

ADMINISTRATOR'S ORDER.

In accordance with Schedule A, Section 5 of this code (a Code of Fair Competition for the Retail Trade, approved by the President on October 21, 1933), the representatives upon the national retail drug trade council may, until such time as the trade associations who presented the code for the retail drug trade shall have submitted for approval a method of electing representatives or until I shall otherwise designate, be those who have already been elected by, or appointed by the board of directors of the AMERICAN PHARMACEUTICAL ASSOCIATION, the Drug Institute of America, Inc., and the National Association of Retail Druggists.

HUGH S. JOHNSON,
Administrator for National Recovery.

WASHINGTON, D. C.
October 24, 1933.

Approved:
A. D. WHITESIDE, *Division Administrator.*
