CONCLUSIONS.

These results show that in the assay of sodium salicylate, sodium benzoate and ammonium benzoate most accurate and uniform results are obtained by either weighing the metal as chloride or extracting the liberated benzoic acid with chloroform and weighing it. If a method of general application is desired, the extraction of the acid and weighing it is the only advisable one. It is quite unlikely that lithium, mercury, or strontium could be weighed as chloride. In addition to its accuracy, Method *2b*, the weighing of the metal as chloride, is exceedingly simple and time saving. Determinations upon the sodium salts by this method require practically no time beyond that of the two weighings necessary.

Acknowledgment is made to Mrs. Frances Stogis of the University of Illinois School of Pharmacy for her careful and conscientious work in making many of the determinations, especially those on sodium salicylate.

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TOXICITY OF QUININE-ASPIRIN MIXTURE ON WARM-BLOODED ANIMALS.*

BY E. A. RUDDIMAN AND C. F. LANWERMEYER.

At the meeting of the ASSOCIATION one year ago, we presented a paper to this section on the Toxicity of Quinine-Aspirin Mixture.[1] The frog was the test-animal in the work reported on at that time. It was suggested that different results might be obtained on warm-blooded animals. This paper presents the results of the work done during the past year on rats and guinea-pigs.

Referring to the report of last year (JOUR. A. PH. A., November 1924, 1009) we find that the minimum fatal dose of the freshly made Quinine-Aspirin Mixture (quinine alkaloid two parts to aspirin three parts) was about 0.00017 gram per gram body-weight of the frog and that the minimum fatal dose of the old Quinine-Aspirin Mixture (after it has changed to the brown-red mass) is 0.00016 gram. There is only a difference of 0.00001 gram, which might easily be due to experimental error on the part of the operator, and the variation of action on the animal.

Consequently, we can say that the old Quinine-Aspirin Mixture is no more toxic for the frog than the freshly prepared mixture.

The animals used this year were white rats and guinea-pigs. The rats were reared in our own laboratory; were all in healthy condition; were between three and four months old and weighed from 61 to 175 grams. Sixty-one rats were

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* Read before Scientific Section, A. PH. A., Des Moines meeting, 1925.

[1] JOUR. A. PH. A., November 1924, p. 1009.

used. The guinea-pigs used were about half grown, weighing from 175 to 300 grams. Sixteen pigs were used.

The Quinine-Aspirin Mixture used was composed of two parts of quinine alkaloid to three parts of aspirin. In this paper, when reference is made to an "old quinine-aspirin mixture" it refers to a mixture which was kept for about a year and a half and had changed to a brown-red mass.

The solvent used was ethylene glycol seventy-five parts and water twenty-five parts. The proportions of quinine to aspirin and of ethylene glycol to water were the same as used last year. At that time it was found that ethylene glycol is one of the best solvents, having but little toxic effect. The toxicity of this compound was further reduced by the addition of 25 per cent of water, which was about the maximum amount of water that could be added without causing precipitation.

The solution used for the rats contained 5 per cent of the Quinine-Aspirin Mixture and the solution for the pigs contained 10 per cent. The stronger solution was used on the larger animal in order to keep the volume of liquid to be injected as small as possible. These solutions were made up fresh, consequently quite a number of solutions were used. The twenty-four hour period of observation was chosen more or less arbitrarily, although if the animal lived twenty-four hours it would generally recover.

In the case of the frog, the solution was injected into the lymph sac under the tongue. In the case of the rats and guinea-pigs the solution was injected hypodermically into the abdomen by means of a tuberculin syringe graduated to 0.01 cc., care being used not to puncture the abdominal cavity.

Solutions of Quinine and Aspirin alone were not used in this series of experiments as our object was to determine the relative toxicity of the fresh and the old mixture.

TABLE I.—EFFECT OF A FRESHLY MADE QUININE-ASPIRIN MIXTURE ON RATS.

Rat no.	Wt. of rat.	Cc. given.	Gm. per Gm. body-weight.	Results after 24 hours.
3	116 Gm.	0.40	0.00017	Alive
4	200 Gm.	0.72	0.00018	Alive
5	217 Gm.	0.83	0.00019	Alive
6	217 Gm.	1.08	0.00025	Alive
7	242 Gm.	1.45	0.0003	Alive
27	136 Gm.	0.82	0.0003	Alive
11	61 Gm.	0.39	0.00032	Alive
28	160 Gm.	1.02	0.00032	Alive
59	112 Gm.	0.72	0.00032	Alive
67	78 Gm.	0.50	0.00032	Alive
74	157 Gm.	1.01	0.00032	Alive
12	76 Gm.	0.50	0.00033	Dead
13	54 Gm.	0.36	0.00033	Alive
60	94 Gm.	0.62	0.00033	Alive
10	60 Gm.	0.41	0.00034	Dead
29	118 Gm.	0.80	0.00034	Dead
61	90 Gm.	0.61	0.00034	Alive
68	105 Gm.	0.72	0.00034	Alive
73	156 Gm.	1.06	0.00034	Alive
9	135 Gm.	0.95	0.00035	Dead

TABLE I.—(*Continued.*)

62	83 Gm.	0.60	0.00036	Alive
69	114 Gm.	0.82	0.00036	Alive
72	150 Gm.	1.08	0.00036	Alive
63	87 Gm.	0.66	0.00038	Alive
66	85 Gm.	0.65	0.00038	Alive
71	138 Gm.	1.05	0.00038	Alive
8	133 Gm.	1.06	0.0004	Dead
64	108 Gm.	0.86	0.0004	Alive
65	85 Gm.	0.68	0.0004	Alive
70	130 Gm.	1.04	0.0004	Alive
75	89 Gm.	0.73	0.00041	Dead
76	85 Gm.	0.72	0.00042	Dead
77	84 Gm.	0.73	0.00043	Alive
78	76 Gm.	0.67	0.00044	Dead
79	101 Gm.	0.89	0.00044	Dead

The minimum fatal dose is approximately 0.0004 gram per gram body-weight, although we find four rats dying within twenty-four hours when smaller doses (0.00033, 0.00034, 0.00034 and 0.00035 gram) were given. These exceptions are not to be wondered at when we consider the fact that in the human being there is a great variation in the amounts of any one medicine which can be taken.

TABLE II.—EFFECT OF AN OLD QUININE-ASPIRIN MIXTURE ON RATS.

Rat no.	Wt. of rat.	Cc. given.	Gm. per Gm. body-weight.	Results after 24 hours.
16	66 Gm.	0.40	0.0003	Alive
18	160 Gm.	0.96	0.0003	Alive
22	144 Gm.	0.86	0.0003	Alive
15	53 Gm.	0.34	0.00032	Alive
23	147 Gm.	0.94	0.00032	Alive
58	115 Gm.	0.78	0.00034	Alive
17	117 Gm.	0.82	0.00035	Alive
24	150 Gm.	1.05	0.00035	Alive
57	83 Gm.	0.58	0.00035	Alive
52	100 Gm.	0.72	0.00036	Alive
25	127 Gm.	0.94	0.00037	Alive
53	93 Gm.	0.69	0.00037	Alive
45	114 Gm.	0.87	0.00038	Alive
51	164 Gm.	1.25	0.00038	Dead
54	91 Gm.	0.69	0.00038	Alive
49	170 Gm.	1.33	0.00039	Dead
55	90 Gm.	0.70	0.00039	Dead
19	123 Gm.	0.98	0.0004	Dead
26	142 Gm.	1.14	0.0004	Alive
44	108 Gm.	0.87	0.0004	Dead
50	130 Gm.	1.04	0.0004	Dead
56	83 Gm.	0.67	0.0004	Alive
80	94 Gm.	0.77	0.00041	Dead
81	88 Gm.	0.74	0.00042	Dead
82	81 Gm.	0.70	0.00043	Dead
83	105 Gm.	0.92	0.00044	Dead

The minimum fatal dose can be placed at about 0.0004 gram of mixture per gram body-weight, two rats having survived and three succumbed to this dose. One died with a dose of 0.00038 and two with a dose of 0.00039. From these

results we came to the conclusion that there is practically no difference in the toxicity of a fresh and an old mixture of Quinine-Aspirin on the white rat.

TABLE III.—EFFECT OF A FRESHLY MADE QUININE-ASPIRIN MIXTURE ON GUINEA-PIGS.

Pig no.	Wt. of pig.	Cc. given.	Gm. per Gm. body-weight.	Results after 24 hours.
4	196 Gm.	0.59	0.0003	Alive
7	308 Gm.	1.08	0.00035	Alive
9	273 Gm.	1.01	0.00037	Alive
6	252 Gm.	0.96	0.00038	Alive
8	206 Gm.	0.81	0.00039	Alive
5	256 Gm.	1.03	0.0004	Dead
22	175 Gm.	0.72	0.00041	Dead
23	238 Gm.	1.00	0.00042	Dead

The minimum fatal dose of the fresh mixture is about 0.0004 gram per gram body-weight.

TABLE IV.—EFFECT OF AN OLD QUININE-ASPIRIN MIXTURE ON GUINEA-PIGS.

Pig no.	Wt. of pig.	Cc. given.	Gm. per Gm. body-weight.	Results in 24 hours.
11	283 Gm.	0.99	0.00035	Alive
15	236 Gm.	0.85	0.00036	Alive
14	262 Gm.	0.97	0.00037	Alive
13	231 Gm.	0.88	0.00038	Alive
12	266 Gm.	1.04	0.00039	Alive
10	248 Gm.	1.00	0.0004	Dead
20	266 Gm.	1.09	0.00041	Dead
21	200 Gm.	0.84	0.00042	Dead

The minimum fatal dose of the old mixture is the same as of the fresh mixture, about 0.0004 gram per gram body-weight.

The conclusion from these experiments is that the old Quinine-Aspirin Mixture is no more toxic on the white rat or guinea-pig than the freshly made mixture.

The difference in the size of the fatal dose for the frog and that for the rat or pig may be due to some extent to the difference of administration. Absorption may not be as fast from hypodermic injection as by injection into the lymph sac.

A 5-grain capsule of the old mixture was given orally to a kitten weighing 297 grams. It lived over the twenty-four hours.

A dog weighing about 9.5 Kg. was given ten 5-grain capsules of the old mixture in a period of twenty-four hours, having been previously starved for twelve hours. He showed no apparent ill effect and ate a hearty meal three hours after the last capsule.

If the effect of these fresh and old mixtures on the human body is similar to that on the lower animals, there is no danger in giving this combination even though it is not absolutely fresh. However, it is probably better to use only the fresh mixture because the aspirin is not then decomposed into its constituents.

In the paper given last year some comments were made on the length of time required for a mixture of quinine and aspirin to become massed and to assume a brown-red color. In January 1924, mixtures were made of aspirin with quinine sulphate, quinine alkaloid, quinine bisulphate, quinine hydrochloride, quinidine sulphate, quinidine alkaloid, cinchonine sulphate, cinchonine alkaloid, cinchonidine

sulphate and cinchonidine alkaloid. Some samples of the alkaloids were first dried, some mixed with citric acid as well as aspirin, some mixtures kept in the light and some in the dark, some kept in tightly sealed containers and some exposed to the air. On examining these mixtures, after standing for a year and a half there is not a sufficient uniformity in results to draw any definite conclusion. In some cases, citric acid seemed to hasten reaction and in others not. The drying of the alkaloid or its salt seemed to retard change to some extent. The mixtures containing free alkaloids started to react more quickly than in case of their salts, but in several instances the massing was not as great at the end of a year and a half.

The change seems to go through the following stages:

> A damp powder, becoming dried and caked, then contracted and of a dirty white color, then a granular mass of dirty yellow-brown color and later a clear mass, too stiff to pour, and of a brown-red color. When the samples had progressed far enough to cake, the odor of acetic acid was quite noticeable, becoming stronger as the reaction went on.

Quinine Sulphate.—All samples had massed except two, in which the salt had been previously dried. The color of most of them was a dirty yellowish gray; none had become brown-red.

Quinine Alkaloid.—All samples had become yellow-brown and had either contracted or had massed.

Quinine Bisulphate.—All mixtures were white, some were in the form of a sticky powder or had caked. None were contracted or massed and all had a little acetic odor.

Quinine Hydrochloride.—The mixtures with starch had not massed; all others had.

Quinidine Sulphate.—All samples had become massed and of a light yellow-brown color.

Quinidine Alkaloid.—All samples had become contracted, but had not massed to any extent. The color was a dirty white. The reaction was not as great as in the case of the sulphate.

Cinchonine Sulphate.—All mixtures had massed. Some had become clear and of a brown-red color, others were granular masses. The results were practically the same as in case of quinidine sulphate.

Cinchonine Alkaloid.—These samples were about the same as those containing the sulphate.

Cinchonidine Sulphate.—The mixtures had not changed as much as those of cinchonine sulphate. Some had massed while quite a number were damp powders.

Cinchonidine Alkaloid.—Reaction seemed to have progressed a little farther than in the case of cinchonidine sulphate.

SUMMARY.

(1) The four cinchona alkaloids and their common salts, when mixed with aspirin and allowed to stand for as much as a year and a half, have changed to a mass. The most pronounced exception to this statement is in case of quinine bisulphate. None of the combinations of this salt had changed more than to a sticky powder, none had gone to the next stage—that of contraction.

(2) A quinine-aspirin mixture, which has been kept until it has changed to a brown-red mass, is no more toxic to several of the lower animals than the freshly made mixture.

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HYDROLYSIS OF THE CRUDE PROTEIN FROM LIQUOR FOLLICULI.*[1]

BY BRYANT FULLERTON AND FREDERICK W. HEYL.

In a recent publication[2] it was noted that the precipitation of liquor folliculi with alcohol yielded crude protein to the extent of 6.34%. This crude protein, which may of course consist of a mixture has been subjected to the Van Slyke process and the nitrogen distribution determined. The tyrosine and tryptophane content has also been determined colorimetrically. The results of our analysis are tabulated below; and also those showing the composition of the albumin of ovarian residue, of mammary gland and casein for purposes of comparison.

	Follicular fluid.	Albumin ovarian residue.	Mammary[3] giand.	Casein.
Total N	100.0	100.00	100.00	100.00
Amide N	8.3	7.80	5.50	10.27
Humin N	1.9	1.44	2.50	1.28
Arginine N	12.6	11.60	11.10	7.41
Cystine N	0.95	0.70	....	0.20
Histidine N	0.00	0.20	4.66	6.21
Lysine N	14.80	14.69	12.47	10.30
Mono Amino N	61.4	60.55	57.17	55.81
Non Amino N	1.5	3.08	6.40	7.13

	Follicular fluid.	Albumin ovarian residue.
Tyrosine	7.1%	8.5
Tryptophane	2.5%	2.1
Molisch test	−	+

In the first place, it is very difficult (except in sulphur content) to decide whether the protein of the follicular fluid is actually different from that of the ovary itself. While the former does not give the Molisch test, the positive test in ovarian protein may be due to absorption of positively testing material. It does not appear that any great alterations of structure, such as are noted in comparing casein and mammary gland, have been produced by the secretory process.

The analysis of mammary gland protein is really not comparable because in this hydrolysis no effort was made to separate the soluble proteins. It appears, however, that the arginine (12.6% N) is very similar to that found in various other organs; the absence of histidine is a conspicuous difference; lysine is somewhat higher than for any other organ protein except placenta.[4]

* From the Chemical Research Laboratory, The Upjohn Company.

[1] Received for publication December 1, 1925.

[2] JOUR. A. PH. A., XIV, 210 (1925).

[3] See Harding and Fort, *J. Biol. Chem.*, 35, 37 (1918).

[4] *J. Biol. Chem.*, 35, 37 (1918).