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Acetanilid Studies. I. Acute Toxicity*

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Acetanilid is a widely used drug, and its derivatives (including sulfanilamide) are increasing annually. A critical survey of the literature revealed many inconsistencies and contradictions with respect to the pharmacology, the pharmacodynamic action and the toxicity of acetanilid, and this investigation was undertaken with the hope of clarifying the situation. This paper deals only with acute toxicity; that is, with the development of death within twenty-four hours or less, after the administration of a known quantity of acetanilid. Some reports have been found on the toxicity of combinations of acetanilid with other medicaments, but this information will be considered in a subsequent paper of this series.

In searching the literature, every effort has been made to consult the original publications, because of the inaccuracies encountered in some abstracts. When possible, contact has been established with the authors of published reports to clear up any uncertainties and to prevent misinterpretation of published statements. In our laboratory studies healthy animals have been purchased from several dealers. Three lots of acetanilid have been used; all conform to the U. S. P. requirements and no significant differences were observed in the pharmacological action of any of these lots. Injections and observations were made by several of the authors throughout the entire study.

LITERATURE SURVEY

Acetanilid was introduced by Cahn and Hepp (9) in 1886. They had intended to use naphthaline as an antipyretic but acetanilid was furnished them instead. After its efficiency as an antipyretic had been established, a series of toxicity tests was started, as well as clinical investigations to determine the usefulness of this product. The material which was used at that time was probably very impure, judging by the present standards for this product. Improvements in method of manufacture have produced chemically pure acetanilid which may be even less toxic than the early product. A compilation of the studies on animals has been prepared in which the largest dose that has been survived by 50% or less of the test animals ($LD_{0\%}$–$LD_{50\%}$) is designated as the "tolerated dose" and the dose which kills all or practically all of the animals tested ($LD_{90\%}$–$LD_{100\%}$) as the "lethal dose." These values are presented in Table I. The data on frogs (7, 21, 43), mice (15, 21, 22, 25), rats (22, 48, 50), guinea pigs (21, 22, 28, 50), rabbits (10, 22, 25, 50, 57), cats (12, 42), dogs (17, 19, 20, 22, 25, 38, 42, 50, 57) and monkeys (47) have been classified in accordance with the reported

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method of administration. The results have been reduced to mg. per Kg. of body weight. In some reports, the solvent or the suspending medium used for the preparation of the test solution has been reported, since it appeared to influence the toxicity findings.

Table I.—Acetanilid: Acute Toxicity. Literature and Laboratory Data

Animal	Method of Administration	Tolerated Dose, Mg./Kg.	Lethal Dose, Mg./Kg.	Remarks
Frogs	Heart perfusion	.....	3500 p. p. m.[a]	Stopped in 95 seconds
Mice	Oral subcutaneous	1840	.....	.....
		1200–1350	1200–1300	55% alcohol
Rats	Oral	400 (LD_0%)	800	LD_{50}%:acacia
	Oral	.....	1200	LD_{100}%:acacia
	Oral	.....	2400	LD_{100}%:50% alcohol
Guinea pig	Oral	.....	1400–1500	.....
Rabbits	Oral	1200	1500	.....
Cats	Oral intravenous	.....	250	.....
		.....	8.0–13.5	.....
Dogs	Oral intravenous	750–1000	700–1000	.....
		62.5–75.0	175–300	.....
Monkeys	Oral intravenous	600	.....	.....
		275	300	.....
Humans	Oral	25–500	5–250	.....

[a] P. p. m. = parts per million.

Table II.—Effect of Oral Administration of Acetanilid on Humans. Literature Data

Survived		Total Dose,	Died		
Age	Sex	Grains	Age	Sex	Remarks
5 mo.	..	3	..	..	
..	..	5	A	F	9 hrs.; no autopsy
..	..	7.5	29	M	4 hrs.; emesis, nephritis
2 wks.	..	10	..	..	
..	..	13.5	7	M	2 hrs.; no autopsy
..	..	18	22	M	10 hrs.; no autopsy
22	F	22.5	..	..	
..	..	25	33	M	1 day; epileptic, chloral, bromides for 1 year
..	..	32	C	..	1 day; no autopsy
A	M	42	..	..	
29	M	45	..	..	
A	M	60	..	..	
A	M	60	..	..	
5	M	60	..	..	
..	..	60	37	M	9 days; nephritis, jaundice
A	M	62	..		
A	M	75	..	..	
34	M	75	..	..	
70	M	75	..	..	
A	M	90	..	..	
75	M	100	..	..	
A	M	105	..	..	
A	M	120	..	..	
A	F	120	..	..	
28	M	120	..	..	
A	M	142	..	..	
A	M	170	..	..	
24	M	180	..	..	
21	M	200	..	..	
..	..	200	A	M	1 day; no autopsy
A	M	300	..	..	
40	M	450	..	..	
20	M	480	..	..	

A = Adult. C = Child. F = Female. M = Male.

Similarly, the specific information in the literature regarding the case reports on acetanilid, most of the very large number of which deal with the administration of less than 40 grains to adults, has not been included. All information dealing with death from any reported amount of acetanilid, and of survival of infants with small doses, or of adults with larger doses, has been collected (1, 2, 3, 4, 5, 6, 8, 13, 14, 16, 18, 21, 23, 26, 27, 30, 31, 32, 33, 34, 35, 36, 37, 40, 44, 45, 46, 51, 53, 54, 55, 56). The data obtained are recorded in Table II. In studying the information presented in Table II,

it may be noted that an infant, aged 2 weeks, survived 10 grains of acetanilid and an infant 5 months old survived 3 grains. In general, the large number of reports of survivals of quantities up to 40 grains were not tabulated. Specific reference is made to 4 individuals surviving doses of 60 grains; the ages of 3 were not reported, but one was a 5-year-old boy. Nine individuals (adults) survived 100 to 200 grains. One instance each was reported of survival after the ingestion of 300 grains, 450 grains and 480 grains of acetanilid by adults.

A total of 8 reports of deaths were found, as indicated in Table II. The quantities of acetanilid taken ranged from 5 grains to 200 grains. In 5 of these cases, the antemortem history was not given and no postmortem was performed, so there is no convincing scientific proof that acetanilid was the cause of death. The death of an adult female within 9 hours after taking 5 grains of acetanilid would appear to be due to idiosyncrasy, in the light of the survival of so many individuals with doses in excess of 100 grains. A man, aged 29, died 4 hours after taking 7.5 grains of acetanilid. The case history showed that he vomited most of the acetanilid immediately after taking it and post-mortem examination showed marked nephritis which might have been sufficient to cause death. An epileptic male, aged 33, who had been taking large quantities of chloral and bromides for a year, died within 1 day after ingestion of 5 grains of acetanilid. No post-mortem examination was made. A man, aged 37, died 9 days after taking 60 grains of acetanilid. Post-mortem examination showed marked nephritis and jaundice. An exhaustive search of the literature has failed to reveal any additional published records of death following the ingestion of acetanilid.

The pharmacological studies by Hale (20) in 1909 suggested that certain combinations of drugs increased the toxicity of acetanilid. Subsequent pharmacological studies indicated that other factors than acetanilid were responsible for the injurious effects noted. A report by Kebler, Morgan and Rupp (26) in 1909 presented opinions on the harmful effects of acetanilid, antipyrin and phenacetin, as indicated in replies to a questionnaire. The authors report a total of 614 cases of poisoning from acetanilid and claim 16 deaths. The original case reports referred to in this bulletin are scientifically unconvincing. Subsequent studies by Helms and Lowy (22, 29, 30) summarized the studies in the United States based on reports obtained from various hospitals over a period of 10 years. Based on a total of 25,000,000 admissions during 10 years, acetanilid was found to be responsible for 5.6 admissions per million and 0.16 death per million admissions. The admissions and the deaths due to barbiturates were ten times as large as those due to acetanilid. A survey of deaths of children 5 years and under in New York State (exclusive of New York City) in the period from 1926 to 1932, inclusive (58), showed a total of 158 deaths, of which one was due to acetanilid.

Herz (24) presented a clinical review in 1934 to show that acetanilid is a safe and useful drug. Sollman (49) states that small doses, such as 3 grains of acetanilid, may be repeated a few times at 3-hour intervals, and Cushny (11) states that acetanilid is not very poisonous. Reid Hunt and Gettler (39) stated that the fatal dose was impossible to state, since death had been reported from

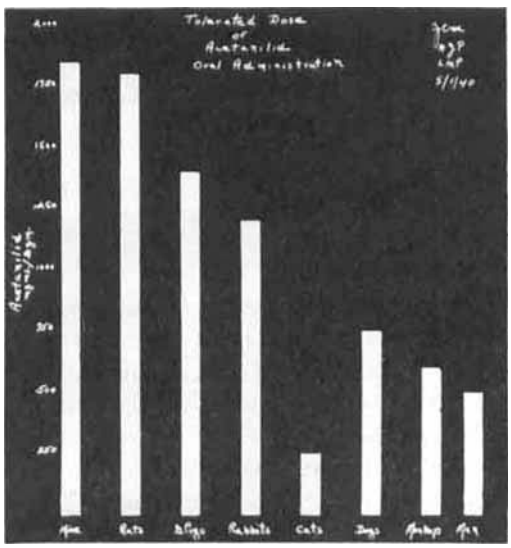

Fig. 1.—Toxicity of Acetanilid to Various Species of Animals—Oral Administration.

8 grains and recovery from doses above 120 grains. Reimann (41) reports that acetanilid produces an untoward effect in degenerative cardio-vascular renal diseases in individuals in doses of 0.6 to 1.3 grams; deaths in apparently normal humans in doses of 0.6 to 2 grams, although recovery followed the oral administration of 4 to 8 grams (60–120 grains). Thienes (52) states that death has followed 0.66 to 2 grams of acetanilid in children, aged or very sick patients; for a healthy adult the acute fatal dose is probably above 30 grams (450 grains). From this collection of information, the data on humans have been assembled as reported in Table I. Figure 1 was prepared to show the toxicity of acetanilid, in mg. per Kg., following oral administration to various species of animals. The ordinates are the maximum tolerated doses reported in Table I, except for rats, in which the average of the two lethal dose values was used, and for guinea pigs and cats in which the lethal doses were used, since the tolerated doses were not known. In our studies, it appeared that the tolerated doses is about 80% of the lethal dose. It is not believed that any significant error is introduced by using these values, to follow the trend of relationship between species and toxicity. Much to our surpirse, there appeared to be a definite progressive increase in toxicity of acetanilid

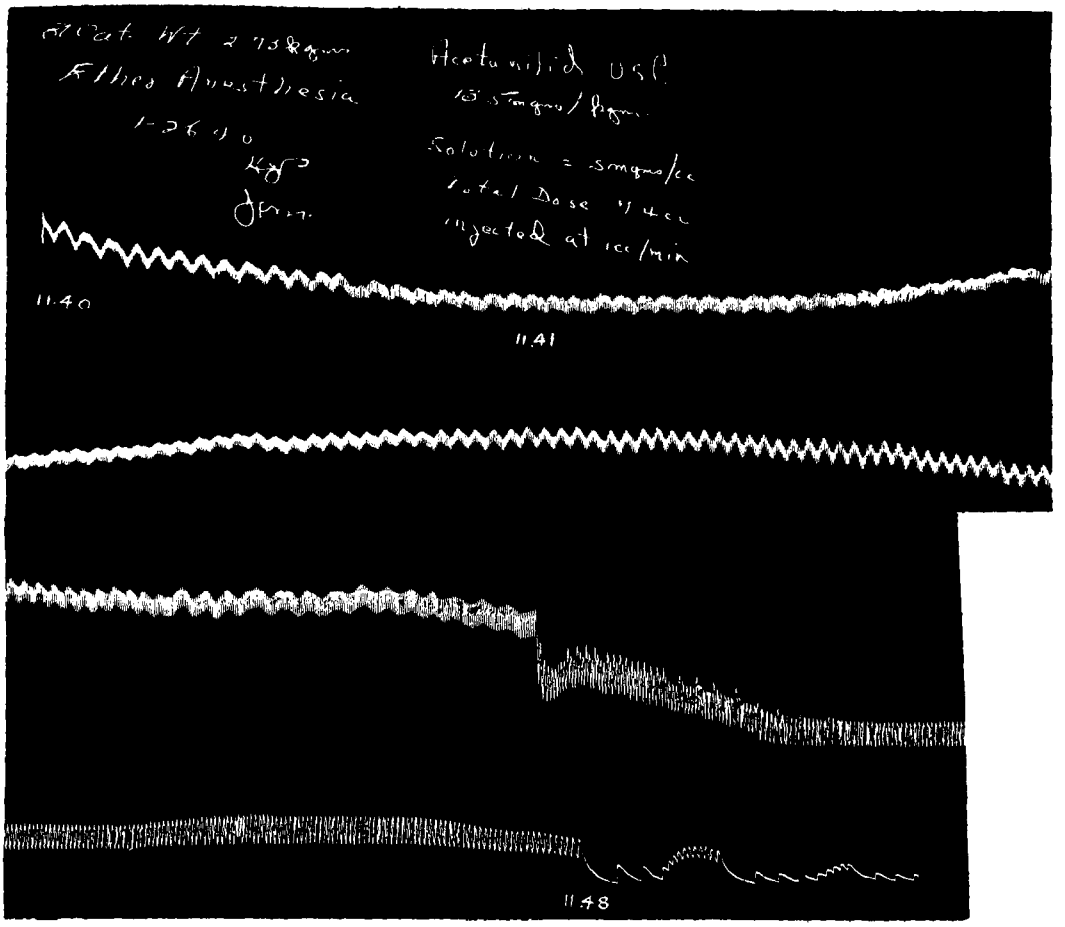

Fig. 2.—Effect on Blood Pressure of Intravenous Injection of Acetanilid to an Anesthetized Cat.

Protocol.—Male cat, 2.73 Kg., etherized, injected with solution containing 5 mg. of acetanilid per cc. in distilled water, at the rate of 1 cc. per minute. Injection was begun at 11:40 A.M., and no alterations in respiration were observed until 11:47 A.M. A slight fall in blood pressure was followed by a slight rise suddenly passing into a drop of 30 mm. at 11:47 and death at 11:48 A.M., January 26, 1940. See Fig. 2.

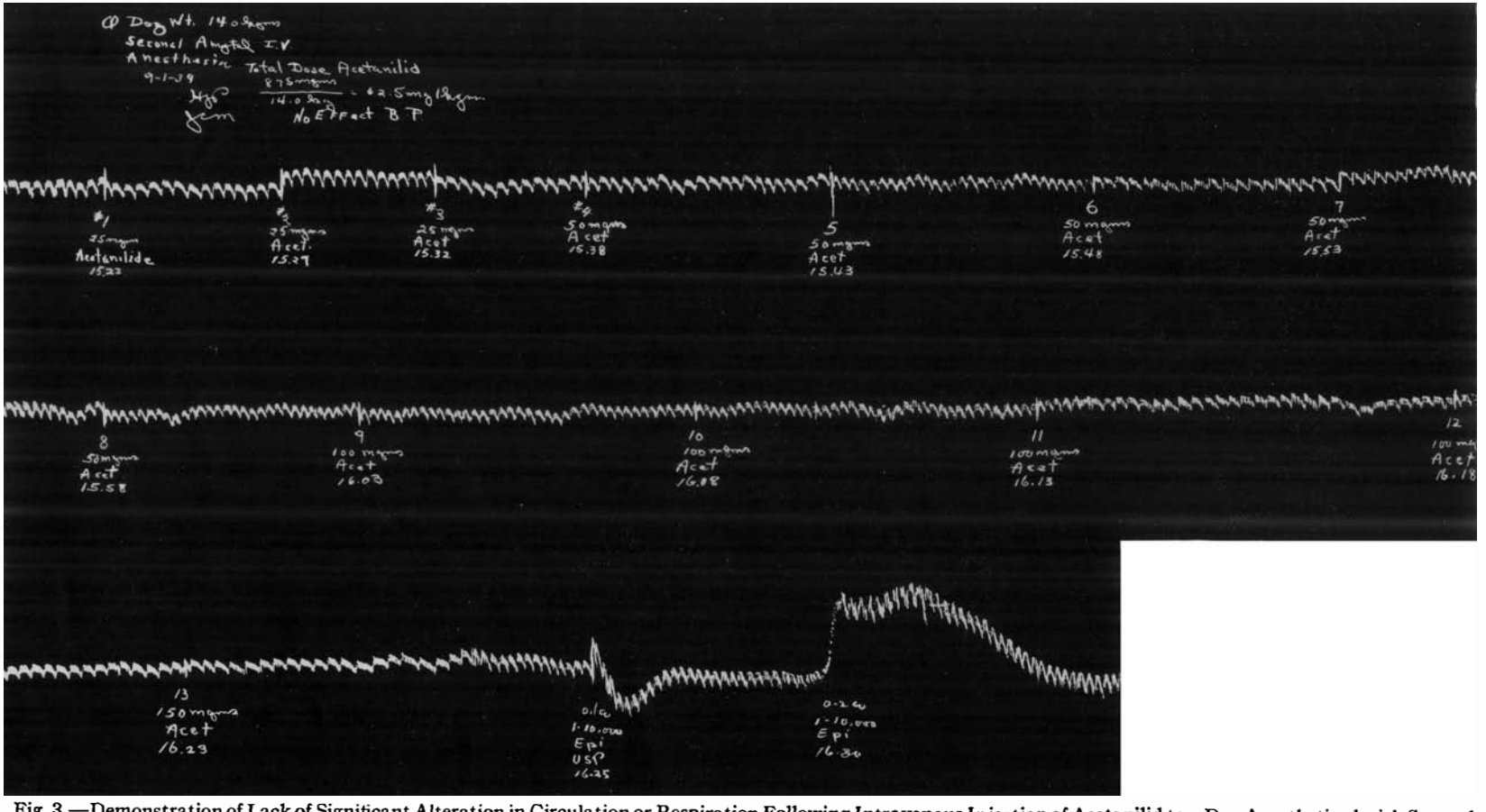

Fig. 3.—Demonstration of Lack of Significant Alteration in Circulation or Respiration Following Intravenous Injection of Acetanilid to a Dog Anesthetized with Seconal.

Protocol.—Female dog, 14 Kg., anesthetized with Seconal I.P., after a series of injections of epinephrine, was injected with a solution containing 5 mg. of acetanilid per cc. in distilled water, at 5-minute intervals. Interrupted tracings of blood pressure were made. A series of three doses of 25 mg. of acetanilid were given, the second of which caused a transient pressor response. A series of 5 injections of 5 mg. each failed to produce significant changes. A series of 4 injections of 100 mg. each caused no significant changes. A single injection of 150 mg. of acetanilid at 16:23 caused a very slight rise in blood pressure. The total dose of acetanilid administered was 875 mg., or 62.5 mg. per Kg. After this series of acetanilid injections, U. S. P. Standard Epinephrine in doses of 10 gamma and 20 gamma produced the same increases in blood pressure as the same doses had produced before injection of acetanilid, September 1, 1939. See Fig. 3.

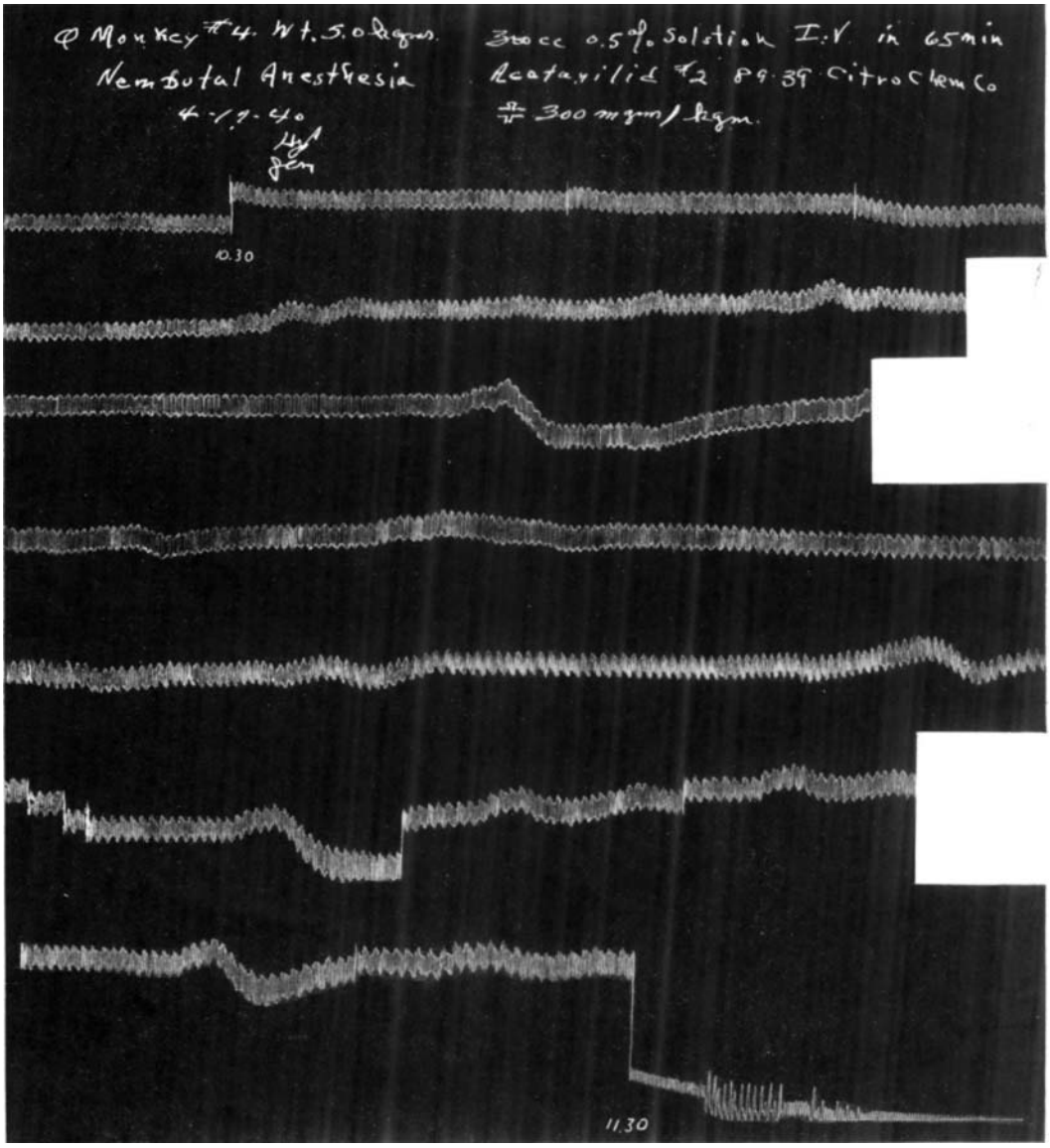

Fig. 4.—Alterations in Blood Pressure Produced by the Intravenous Injection of Acetanilid to a Monkey Anesthetized with Nembutal.

Protocol.—Female monkey, 5 Kg., anesthetized with Nembutal I.P., injected with solution containing 5 mg. of acetanilid per cc. in distilled water continuously. The first injection at 10:30 A.M. caused a prompt rise in blood pressure of about 25 mm., which gradually returned to the normal level. Slight but not significant rises and falls in blood pressure were observed during the series of injections until 11:15 when a total fall of about 20 mm. developed, followed by a rather rapid return to the normal blood pressure level. At 11:28 there was a sudden rise followed by a fall of equal magnitude, and leading to the original blood pressure again. At 11:30 there was a sudden precipitous drop of 55 mm. in blood pressure, with apnea. Death developed within 5 minutes or before 11:35 A.M., April 17, 1940. The total dose administered was 1500 mg., or 300 mg. per Kg. See Fig. 4.

when administered orally to animals, in the order of mice, rats, guinea pigs, rabbits, dogs, monkeys, man. Cats are definitely out of line in this progression, being about twice as susceptible as man. For this reason it is felt that toxicity studies conducted on dogs or monkeys are more significant for application to man than are studies on cats.

LABORATORY STUDIES

Because of the variations in tolerated and in lethadoses for animals and for man obtained in the literal ture survey, laboratory investigations were under-

taken on animals and on a group of humans. Most of our animal studies were made on cats, dogs and monkeys. Typical protocols and records of effect on the blood pressure are shown in Figs. 2, 3 and 4.

Figure 2 shows the effect on blood pressure of intravenous injection of acetanilid to a cat under ether, and the details of the experiment, which is typical of a number of cat tests, are given in the protocol. The lethal dose of acetanilid ranged from 8.5 to 13.5 mg. per Kg. of body weight, to cats anesthetized with ether.

Figure 3 and its protocol show the lack of significant alteration in circulation or respiration following intravenous injection of acetanilid to a dog anesthetized with Seconal. This animal received a total of 875 mg., corresponding to 62.5 mg. per Kg, which did not cause death.

Figure 4 and its protocol show the alterations in blood pressure produced by the intravenous injection of a total of 1500 mg. of acetanilid to a 5-Kg. female monkey, anesthetized with nembutal. The lethal dose was 300 mg. of acetanilid per Kg. of body weight, and death was produced within an hour. No significant alterations in blood pressure or respiration were observed until shortly before death.

DISCUSSION

The question has frequently been asked whether acetanilid is a "poison." The senior author has proposed that a poison might be defined as: "A substance which chemically reacts with normal living tissues with the usual effect of injuring health or destroying life." From a consideration of the information published in the literature, as well as that obtained in our laboratory studies, it does not appear that acetanilid is very poisonous, considered from the standpoint of acute toxicity. The chronic toxicity will be considered in a subsequent report.

CONCLUSIONS

1. The acute toxicity of acetanilid has been determined by literature search and laboratory studies.

2. Following oral administration, there is a progressive increase in susceptibility from mice to rats, guinea pigs, rabbits, dogs, monkeys and man. Cats are out of line, being about twice as susceptible as man. In toxicity studies, having a bearing on human interpretations, dogs and monkeys preferred.

3. No significant effects on blood pressure or respiration were observed, following intravenous injection of acetanilid solution to dogs or monkeys, until the lethal dose was approached.

4. Doses of acetanilid up to 480 grains were tolerated, corresponding to tolerated doses of 25 to 500 mg. per Kg. for humans. Eight deaths were reported in humans, following quantities ranging from 5 to 200 grains, but the lack of post-mortem examinations in 5, and the pathological conditions observed in the other 3 make it difficult to conclude that acetanilid was the cause of death.

5. Considered from the standpoint of tolerated and lethal doses for animals and man, the acute toxicity of acetanilid is relatively very low.

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Certain Salts of Atropine, Ephedrine, Epinephrine and Procaine*

By Frank M. Goyan and T. C. Daniels

It is often desirable to reduce the acidity of solutions of the hydrochlorides, hydrobromides and sulfates of physiologically active bases. In many cases this adjustment might be avoided if salts of weak acids were available to replace the salts of highly ionized acids now in use.

A recommendation that any particular salt is suitable cannot be made on the basis of the p_H of the solution alone but must, of course, await careful pharmacologic and clinical evaluation. This is shown by the work of Régnier and co-workers (1, 2, 3) who report different physiologic activity for various salts of the same base, and by Tainter, Throndson and Moose (4) who have made a careful clinical investigation of the relative merits of procaine borate as compared with procaine hydrochloride.

The report of Stover and Brigham (5) on oil-soluble procaine salts supports the conclusion that significant advances may be made by investigating different salts of the same base. This point is further emphasized by the work of Abderhalden and Vlassopoulos (6) who have shown that the presence of amino acids and polypeptides increases the physiologic activity of ephedrine and epinephrine, thus indicating the possible usefulness of salts prepared by neutralizing these bases with suitable amino acids.

A useful salt of any physiologically active base must also satisfy the requirement of stability, either in the solid state or in aqueous solution or, preferably, both. Aqueous solutions of ester-type compounds, unless stabilized, may be expected to undergo decomposition in neutral or alkaline solu-

* Contribution from the College of Pharmacy, University of California.