STUDIES ON THE HEMORRHAGIC SWEET CLOVER DISEASE

XIV. HYPERPROTHROMBINEMIA INDUCED BY METHYLXANTHINES AND ITS EFFECT ON THE ACTION OF 3,3'-METHYLENEBIS-(4-HYDROXYCOUMARIN)*

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(Received for publication, September 7, 1944)

Several observations have been recorded which suggest that the methylxanthines have an effect on blood coagulation. Klemperer in his treatise, "Untersuchungen ueber Gicht," published in 1896, expressed the thought that caffeine evoked the development of a coagulative ferment (1) p. 59). In 1920 Nonnenbruch and Szyszka suggested that the methylxanthines caused an increase in fibrin ferment (2). Subsequently Meissner (3) and Addicks (4) reported that administration of theophylline shortened the coagulation time of whole blood. The authoritative review by Morawitz on blood and blood diseases (5) and the monograph by Pickering (6) p. 213 cite the use of a preparation composed of theophylline and ethylenediamine in equal proportions as a hemostatic agent. More recently Tobitani (7) reported that substances containing the guanidine nucleus decrease the blood clotting time by increasing the formation of thrombin, while Sirasaka (8) has indicated that the administration of caffeine to rabbits shortened the bleeding time.

The purpose of this report is 2-fold: first to show that the methylxanthines, caffeine (1,3,7-trimethylxanthine), theobromine (3,7-dimethylxanthine), and theophylline (1,3 dimethylxanthine), induce in the dog, rat, and rabbit a state of hyperprothrombinemia as reflected by shortened plasma prothrombin times; secondly, to indicate that, as a result of the induced hyperprothrombinemia, the hypoprothrombinemic action of the anticoagulant 3,3'-methylenebis(4-hydroxycoumarin) is lessened (9).

* Published with the approval of the Director of the Wisconsin Agricultural Experiment Station and supported since July, 1940, through special grants from the Graduate Research Committee of the University, Office of Dean E. B. Fred, and the Wisconsin Alumni Research Foundation. Part of the work embodied in this paper is taken from a thesis by John B. Field presented to the Faculty of the Graduate School in partial fulfilment of the requirements for the degree of Doctor of Philosophy, May, 1944.

1 The basic observations recorded here have been known to us since May, 1942. In March, 1943, a collation of the findings was circulated privately among certain
Methods

Previous publications give all the basic techniques employed as well as the rationale of handling the animals. Specific citations are made when necessary. In this study, as in all the previous work (10–12), whole plasma (100 per cent) and the 50, 25, 12.5, and 6.25 per cent plasma concentrations were routinely explored for prothrombin level (or activity); but since the change in prothrombin activity reflected by the 12.5 per cent plasma (1 part plasma, 7 parts saline solution) is readily detected and reproducible, most of the data are given only in terms of this value.

It should be emphasized that a marked prolongation of the prothrombin time (hypoprothrombinemic state) is possible with the anticoagulant 3,3'-methylenebis(4-hydroxycoumarin) ((11) pp. 950–953). In contrast, the extent to which the prothrombin time can be shortened from the normal by the methylxanthines (hyperprothrombinemic state) is limited. Although the reduction in seconds from the normal is of a small order, the decrease is readily reproducible. Prothrombin concentration varies hyperbolically with shift in prothrombin time in the particular time range in which the hyperprothrombinemia is measured. Hence a large increase in prothrombin concentration may produce only a small change in prothrombin time (see (10) Fig. 1 and pp. 8–10).

The status of our knowledge on prothrombin activity (or concentration) as reflected by readings in seconds does not permit of a direct evaluation of decreased as opposed to increased prothrombin times. A decrease of 2 to 4 seconds from the normal prothrombin time of 12.5 per cent plasma might conceivably involve an actual change in prothrombin concentration (or activity) equivalent to that which occurs when the time is prolonged 20 to 40 seconds above the normal.

EXPERIMENTAL

Hyperprothrombinemic Action of Methylxanthines

Conditions of the Experiments—The dogs were maintained on a diet of skim milk powder 40, yellow corn 15, meat scraps 15, wheat bran 10, wheat middlings 10, alfalfa meal 7, bone meal 2, and salt 1. They were not fasted either before or during the test period. The rats were handled exactly as previously (12), which includes a 12 hour fast before the methylxanthine was given; the rabbits as in (10, 11), except that they were not fasted.

Effect of Single Oral Dose of Theobromine on Plasma Prothrombin Time—

clinical investigators studying the action of 3,3'-methylenebis(4-hydroxycoumarin) in man. The first public report was made for us on February 6, 1944, by Dr. Shepard Shapiro in the seminar on blood coagulation that he conducted in the Department of Therapeutics, New York University.
Results from many trials will be reported in a highly condensed form. A representative response for dog plasma is given in the dilution curves of Fig. 1 and the curve labeled "Theobromine" of Fig. 2. Curve A of Fig. 1 gives the prothrombin times of the normal plasma, Curve B of plasma from the same dog 48 hours after feeding 200 mg. per kilo of theobromine. The shortened prothrombin times over the whole range of the dilution curve are apparent. Comparable responses are realized when caffeine or theophylline is given.  

All three methylxanthines evoke shortened prothrombin times of the same general order when given orally to dogs, rabbits, and rats (Table I).  

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Fig. 1. The effect of theobromine on the prothrombin time of dog plasma. Curve A represents the normal plasma; Curve B, plasma from the same dog 48 hours after feeding theobromine (200 mg. per kilo).
Fig. 2. Representative chart of the protective action of theobromine against 3,3'-methylenebis(4-hydroxycoumarin) in the dog. Theobromine, 100 mg. per kilo, and 3,3'-methylenebis(4-hydroxycoumarin) 10 mg. per kilo, given orally.

**TABLE I**

*Representative Effect of Caffeine on Prothrombin Time of 12.5 Per Cent Plasma of Various Species*

<table>
<thead>
<tr>
<th>Species</th>
<th>Caffeine fed†</th>
<th>Prothrombin time in sec. of 12.5 per cent plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg. per kg.</td>
<td>Normal prothrombin time</td>
</tr>
<tr>
<td>Dog</td>
<td>50</td>
<td>30.4 ± 0.6†</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>29.0 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>22.5 ± 0.4</td>
</tr>
<tr>
<td>Rat</td>
<td>4</td>
<td>40.0 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>37.9 ± 0.5</td>
</tr>
<tr>
<td>Rabbit</td>
<td>100</td>
<td>30.2 ± 0.4</td>
</tr>
</tbody>
</table>

* Each line of figures is a series of values obtained with a single animal.
† As caffeine citrate.
‡ Standard deviation values.

The degree of the response varies in extent and duration, depending on the dose (Table II). Individual variation between members of the same
species is detectable. There is also a variation in response between species. In sum the detectable dose under our conditions in a 250 to 300 gm. rat is 1 to 2 mg., in the rabbit 10 to 25 mg. per kilo, in the dog 25 to 50 mg. per kilo.

Effect of Repeated Doses of Theophylline on Prothrombin Time of 12.5 Per Cent Dog Plasma—Aminophylline (theophylline-ethylenediamine) was fed three times daily to dogs at the 4, 8, and 12 mg. per kilo level. This dosage level is equivalent to the range recommended in Osler-Christian for clinical practice (14). The hyperprothrombinemic effect usually became detectable in 2 to 5 days at the levels indicated. Individual dogs have been maintained in the hyperprothrombinemic state with these levels for periods up to 30 days. During the first 2 weeks the intensity of the response was relatively uniform. There followed then a period with detectable fluctuations. A typical protocol of a dog receiving 12 mg. per kilo of aminophylline thrice daily is given in Table III.

Action of Other Purines, Pyrimidines, and Related Substances—In the dog and rabbit, xanthine (2,6-dioxypurine) in doses up to 200 mg. per kilo was found to be devoid of any hyperprothrombinemic action. Eight out of a group of twenty-two rats showed a border line response at the 100 mg. level. Heteroxanthine (7-methylxanthine) showed slight activity in the rat at 100 mg. None of the following showed activity in doses of

a Synthesized for us here by Mr. Saul Roseman.

TABLE II

<table>
<thead>
<tr>
<th>Theophylline fed† mg. per kg.</th>
<th>Prothrombin time in sec. of 12.5 per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal prothrombin time</td>
</tr>
<tr>
<td>10</td>
<td>30.8 ± 0.6†</td>
</tr>
<tr>
<td>50</td>
<td>32.0 ± 0.7</td>
</tr>
<tr>
<td>75</td>
<td>27.0 ± 1.2</td>
</tr>
<tr>
<td>200</td>
<td>30.0 ± 1.3</td>
</tr>
<tr>
<td>400</td>
<td>31.2 ± 0.5</td>
</tr>
</tbody>
</table>

* Each line of figures represents a series of values obtained with a single dog.
† As theocine (theophylline and sodium acetate).
‡ Standard deviation values based on not less than four and usually more than ten values, obtained over a period of 2 to 3 months with the same dog. Dogs maintain relatively constant prothrombin times over prolonged periods under ordinary conditions (13).
200 mg. per kilo in either the rat, dog, or rabbit; uric acid, adenine, allantoin, urea, creatine, creatinine, guanidine, glycocyamine, arginine, uracil, 6-methyluracil, 2-methyl-5-ethoxymethyl-6-oxypyrimidine.4

Evidence That Hyperprothrombinemic Effect of Active Methylxanthines Is Not Due to Hemoconcentration—Since diuresis is one of the effects that may be evoked by theophylline and caffeine, the urine output of the dogs was measured under the conditions of the tests. The 24 hour average excretion during a 3 to 5 day period, prior to the administration of the methylxanthine, was first ascertained. The drug was then fed at the levels at which it produced a readily detected hyperprothrombinemia. No increase in the pre-test urine excretion was noted. This is in agreement with previously recorded observations that the methylxanthines are usually not effective as diuretics in normal non-edematous animals ((15) p. 638). It has also been indicated that the main diuretic effect of theophylline is usually dissipated within a few hours after administration (16). Furthermore, it has been recorded that theophylline, rather than causing dehydration of the blood stream, actually produces a slight rise in plasma volume (17).

For control purposes the following pertinent determinations were made on dog blood at the plateau of the hyperprothrombinemic response; total plasma protein (18), total plasma nitrogen (19), hemoglobin, and hematocrit (20). No increase in any of these constituents was noted.

As additional evidence that the hyperprothrombinemic effect is not due to diuresis, the action of ammonium chloride, sodium acetate, and

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4 The last two compounds were kindly supplied by Dr. R. T. Major, Research Department, Merck and Company, Inc., Rahway, New Jersey. Professor Henry Borsook, California Institute of Technology, Pasadena, supplied the glycocyamine.
urea was studied. While these substances produced a measurable diuresis, only an occasional and transitory hyperprothrombinemia was noticeable 12 hours after administration. Colossal doses of these substances (1 gm. per kilo of urea and 800 mg. per kilo of the inorganic salts) did not induce a consistent or prolonged hyperprothrombinemia. In contrast the methylxanthines do so when the dose is relatively small.

Evidence That Hyperprothrombinemia Induced by Methylxanthines Is Not Due to Vitamin K-Like Action—The methylxanthines were tested for antihemorrhagic potency (vitamin K activity) by the procedure of Almquist and Klose (21). 1 day-old chicks were fed a vitamin K-deficient diet, to which the methylxanthines had been added at the level of 10 and 100 mg. per kilo of ration. The clotting time of the whole blood, as well as the prothrombin time of the chick plasma, was determined after 1, 2, and 3 weeks. While the addition of 10 mg. per kilo of 2-methyl-1,4-naphthoquinone (menadione, Abbott) to the ration reduced the clotting time of whole blood to 3 to 4 minutes, the whole blood clotting time of the chicks receiving the methylxanthines remained at 20 minutes. Since the assay for antihemorrhagic potency is subject to wide variations, we asked Dr. Carl Nielsen and Mrs. F. P. Dann of the Abbott Laboratories, North Chicago, Illinois, to make control tests for us by the method that they use routinely (22). They also realized negative results with the methylxanthines. Topelberg and Honorato (23) have reported that caffeine does not decrease the prolonged clotting time of chicks deprived of vitamin K.

Effect of Methylxanthines on Action of 3,3'-Methylenebis(4-hydroxycoumarin)

Effect of Methylxanthines on Action of 3,3'-Methylenebis(4-hydroxycoumarin) in the Dog—The individual response of 30 dogs of varying age, weight, and breed to the action of the anticoagulant was first ascertained. The detectable dose of the anticoagulant in the dogs used in these trials under the conditions of the standardization (no fasting), is approximately 2.5 mg. per kilo. The actual dose of the anticoagulant used varied from 7.5 to 15 mg. per kilo. For each dog an individual response curve comparable to that given in ((11) Fig. 3, p. 945) was constructed with whole plasma as well as plasma of various dilutions. But the presentation of the data will be restricted to the 10 mg. per kilo dose of the anticoagulant. It should be emphasized that at this dosage level 3,3'-methylenebis(4-hydroxycoumarin) is capable of inducing a marked hypoprothrombinemia, representing a prolongation of the 12.5 per cent prothrombin time from the average normal value of 25 seconds to 100 to 110 seconds in 48 to 72 hours. Normal prothrombin times were usually restored in from 5 to 7 days.
The dose of methylxanthine varied from 10 to 200 mg. per kilo. Representative responses of individual dogs to the simultaneous administration of anticoagulant and methylxanthines\(^5\) are given in Table IV and Fig. 2, curve labeled "Anticoagulant + theobromine."

**Duration of Protective Effect of Methylxanthines against Action of 3,3'-Methylenebis(4-hydroxycoumarin) in the Dog**—The individual hypoprothrombinemic response evoked by a standard dose of the anticoagulant in mature dogs, rabbits, and rats is quite uniform on a given diet (10–12). It was therefore surprising to find that after a methylxanthine is administered to dogs simultaneously with the anticoagulant, the response to repeated standard doses of the anticoagulant (10 mg. per kilo) is altered. A representative example of the capacity of theobromine to impart a sustained resistance in the dog is illustrated in Table V.

<table>
<thead>
<tr>
<th>Dose of methylxanthine per kilo</th>
<th>Prothrombin time in sec. of 12.5 per cent plasma*</th>
<th>Dose of methylxanthine per kilo</th>
<th>Prothrombin time in sec. of 12.5 per cent plasma*</th>
</tr>
</thead>
<tbody>
<tr>
<td>25 mg. theophylline</td>
<td>64</td>
<td>100 mg. per kilo anticoagulant* +</td>
<td>53</td>
</tr>
<tr>
<td>200 mg. theophylline</td>
<td>76</td>
<td>methylxanthine (control)</td>
<td>26</td>
</tr>
<tr>
<td>10 mg. theobromine</td>
<td>102</td>
<td>10 mg. anticoagulant* +</td>
<td>90</td>
</tr>
<tr>
<td>100 mg. theobromine</td>
<td>89</td>
<td>methylxanthine (control)</td>
<td>84</td>
</tr>
<tr>
<td>10 mg. caffeine</td>
<td>95</td>
<td>10 mg. anticoagulant* +</td>
<td>106</td>
</tr>
<tr>
<td>100 mg. caffeine</td>
<td>106</td>
<td>methylxanthine</td>
<td>67</td>
</tr>
</tbody>
</table>

* The average normal 12.5 per cent prothrombin time is 25 seconds, range 22 to 29 seconds.

The duration of this unique protective effect is dependent on the dose of methylxanthine originally administered. In dogs receiving 50 or 100 mg. per kilo of either caffeine, theobromine, or theophylline, the resistance persisted for approximately 14 weeks; at the 10 to 25 mg. per kilo level, 6 to 8 weeks. Xanthine, at the 100 mg. per kilo level, gave a slight protection, but the resistance usually disappeared within 3 weeks.

**Effect of Methylxanthines on Action of 3,3'-Methylenebis(4-hydroxycoumarin) When Given after Anticoagulant in the Dog**—When methylxanthines are given to dogs in the process of developing the hypoprothrombinemic

\(^5\) A border line protective action by xanthine was noted at the 100 mg. per kilo level. Allantoin, uric acid, urea, and guanidine showed only a slight but variable protective action at levels up to 100 mg. per kilo.
state 24 hours after the anticoagulant has been given, there not only results a reduction in the intensity of the hypoprothrombinemia but the duration is also shortened (Fig. 2). It is to be noted that the effect of the methylxanthine does not become detectable until 24 hours after its administration. If the methylxanthine is given 48 hours after the anticoagulant, the capacity to arrest the developing hypoprothrombinemia is nullified and there appears to be no effect on its duration. The curve in Fig. 2 (labeled “Anticoagulant + theobromine 24 hrs. apart”) was constructed from sixteen separate trials with eight different dogs, in which the anticoagulant was first given at the level of 10 mg. per kilo and theobromine at the 100 mg. per kilo level.

**Table V**

*Resistance to 3,3'-Methylenebis(4-hydroxycoumarin) Caused by Single Dose of Theobromine in a Dog*

<table>
<thead>
<tr>
<th>Date</th>
<th>Treatment</th>
<th>Prothrombin time* (12.5 per cent)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>48 hrs. after administration</td>
</tr>
<tr>
<td>1943</td>
<td></td>
<td>sec.</td>
</tr>
<tr>
<td>Jan. 10</td>
<td>10 mg. anticoagulant per kilo</td>
<td>89</td>
</tr>
<tr>
<td>&quot; &quot; 24</td>
<td>&quot; &quot; &quot; + 100 mg. theobromine per kilo</td>
<td>31</td>
</tr>
<tr>
<td>Feb. 7</td>
<td>10 mg. anticoagulant per kilo</td>
<td>45</td>
</tr>
<tr>
<td>&quot; &quot; 14</td>
<td>&quot; &quot; &quot; &quot; &quot; &quot;</td>
<td>48</td>
</tr>
<tr>
<td>Mar. 7</td>
<td>10 &quot; &quot; &quot; &quot; &quot; &quot;</td>
<td>33</td>
</tr>
<tr>
<td>&quot; &quot; 21</td>
<td>15 &quot; &quot; &quot; &quot; &quot; &quot;</td>
<td>37</td>
</tr>
<tr>
<td>May 23</td>
<td>15 &quot; &quot; &quot; &quot; &quot; &quot;</td>
<td>66</td>
</tr>
<tr>
<td>July 6</td>
<td>15 &quot; &quot; &quot; &quot; &quot; &quot;</td>
<td>77</td>
</tr>
</tbody>
</table>

* The average normal 12.5 per cent plasma prothrombin time of this dog was 25 (±2) seconds at each date prior to the test.

**Effect of Daily Ingestion of Methylxanthines on Survival Time of Rats Receiving 3,3'-Methylenebis(4-hydroxycoumarin)**—The conditions for the survival tests are essentially those given in previous papers in this series (12, 24). The results of many trials are herein summarized. The control rats maintained on the semisynthetic diet (12) survived a daily intake

* The effect of the substances listed below on the action of the anticoagulant in the rat was also tested. They were either given with the anticoagulant or 12 hours later at the 50 or 100 mg. level. Adenine, uric acid, allantoin, xanthine, arginine, xanthopterin, guanidine, methylguanidine, dimethylguanidine, urea, creatine, creatinine, glycocyamine, uracil, and 6-methyluracil. Some of these compounds afforded a detectable protection against the induced hypoprothrombinemia, but their activity does not approach that of theobromine, caffeine, and theophylline.
of 2 mg. of the anticoagulant for about 13 to 15 days. When 100 mg. of theobromine were added to the ration in addition to the anticoagulant, the average survival time was more than 60 days. With a daily intake of 50 mg. of caffeine the survival time was also over 60 days. In sharp contrast, theophylline at the 50 mg. level offered only slight protection, the survival time being 17 days. Likewise xanthine at the 100 mg. level showed only a slight protection. The effect of uric acid, arginine, allantoin, urea, and guanidine was also tested. These compounds are devoid of the capacity to influence survival when the anticoagulant is ingested daily.

DISCUSSION

Control trials indicated that the methylxanthines do not affect the prothrombin time when added to blood or plasma in vitro. The hyperprothrombinemic effect is not due to hemoconcentration resulting from diuresis, or to replacement of the antihemorrhagic naphthoquinones in the synthesis of prothrombin. It would therefore appear that the capacity to elaborate prothrombin must be affected directly. The most plausible rationalization that can be advanced is that a functional stimulation of hepatic tissue is brought about, just as in a contrary manner the action of 3,3′-methylenebis(4-hydroxycoumarin) most likely involves a functional inhibition of prothrombin synthesis.

This point of view has arisen from the study of certain other factors that affect the coagulation mechanism in vivo. Trials with the dog (unpublished) indicate that the methylxanthines reduce the hepatotoxic action of chloroform as reflected by the hypoprothrombinemia measured with 12.5 per cent plasma. Furthermore, we have found that the action of the methylxanthines in normal dogs is not restricted to increased prothrombin activity. A readily detectable increase in the plasma fibrinogen level also results. A full report of this work will follow.

The specific hyperprothrombinemic action of the multimethylated xanthines (caffeine, theobromine, and theophylline), as opposed to the inactivity of related purine derivatives (xanthine, uric acid, adenine, allantoin, glycocystine, urea, creatinine, methylguanidine, and dimethylguanidine), merits comment. The possibility that the activity of caffeine, theobromine, and theophylline might result from a release of methyl groups was explored (25). The administration of massive doses of choline and methionine to dogs (200 to 600 mg. per kilo) alone, or together with large doses of xanthine, was tried. A significant effect on the prothrombin time was not observed. Similar trials with the rat were also essentially negative. In sum, the intact multimethylated xanthine molecule must be available for effective hyperprothrombinemic action. The border line

7 The rats were in good condition and showed no hemorrhagic lesions when the test was terminated.
response realized with heteroxanthine (7-methylxanthine) tends to sustain this premise.

One of the significant points that has been established since the anticoagulant 3,3'-methylenebis(4-hydroxycoumarin) has become available is that a primary relationship exists between thrombus formation and the clotting mechanism of the blood. The work of Dale and Jaques (26), Richards and Cortell (27), Bollman and Preston (28), and Meyer and coworkers (29) on experimental thrombosis done with 3,3'-methylenebis (4-hydroxycoumarin) established that effective reduction of extravascular and intravascular thrombus formation parallels the diminished plasma prothrombin level (or activity) with its associated hypocoagulability.

The methylxanthines are extensively used in the treatment of cardiovascular disorders in man in which the threatening complication is frequently thrombus formation (14, 30). The dose of methylxanthine used is of a relatively high order and it is recommended that they be given thrice daily and frequently for as long as 1 to 2 weeks continuously (Osler-Christian (14)). Since our results on such diverse species as the dog, rabbit, and rat indicate that the methylxanthines not only render the blood hypercoagulable, but also counteract such a potent hypoprothrombinemic agent as 3,3'-methylenebis(4-hydroxycoumarin), it is conceivable that their use in man might augment the tendency for thrombus formation.

We feel that the reported findings merit consideration, especially since it was recently stated (32) in a frank evaluation of drugs used in the treatment of cardiovascular disorders, "It is evident then that there is considerable doubt as to whether or not aminophylline is of value as a coronary vasodilator in man. However, no investigator has found the drug to be dangerous when given by mouth, so it can, therefore, be safely assumed that even though the aminophylline may do the patient no good, it can certainly do him no harm." In the light of the findings presented the justification for this point of view may be questioned (33).

We wish to thank Dr. Carl Nielsen and Mrs. F. P. Dann, Abbott Laboratories, North Chicago, for independently assaying the methylxanthines for antihemorrhagic activity in the chick and Dr. R. S. Overman for collaborating with us in the early stages of this study.

SUMMARY

1. It is shown that single oral doses of the methylxanthines, theophylline, theobromine, and caffeine, induce in the dog, rabbit, and rat a state of

8 The eminent pathologist Aschoff has written, "The view that increased coagulability of blood is an essential point for the production of thrombosis, has been strongly upheld, especially by clinical observers. The existence of this increased coagulability and the likelihood that it is a promoting factor, or, better, an accompanying phenomenon of thrombosis cannot be denied." (31) pp. 253-254.)
hemorrhagic sweet clover disease. XIV

hyperprothrombinemia. This effect is not exhibited by other purines, pyrimidines, and related compounds. Choline and methionine are likewise devoid of this action.

2. The hyperprothrombinemia resulting from a single dose of methylxanthine persists for 4 to 5 days in the dog. Through repeated small doses dogs were maintained in the hyperprothrombinemic state for periods up to 30 days.

3. The methylxanthines can counteract the hypoprothrombinemic action of the anticoagulant 3,3'-methylenbis(4-hydroxycoumarin) in the dog. When they are given either with, or 24 hours after, the anticoagulant, they not only reduce the intensity of the hypoprothrombinemic response but also shorten its duration.

4. Single doses of the methylxanthines will protect a standardized dog against repeated doses of the anticoagulant for periods up to 14 weeks. Continued ingestion of caffeine and theobromine prolonged the survival time of rats fed the anticoagulant daily.

5. It is suggested that the methylxanthines produce a functional stimulation of hepatic tissue, which accounts for the hyperprothrombinemia in normal animals and for the protective action against the anticoagulant.

6. A possible bearing of these findings on the use of methylxanthines in conjunction with cardiovascular therapy is suggested.

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J. Biol. Chem. 1944, 156:725-738.

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