possibility that simple incubation of estrone in blood will lead to degradation to inactive compounds as well as conversion to the new compound suggested. Previous determinations on short-time incubation of estrone in blood have led to recoveries approaching 100% of the activity added. Such tests were made both by us and by Pincus and Schiller (*6*).

It therefore appears reasonable to assume from these data that an alcoholic ketone derivative of estrone is normally present in human and rabbit blood.

It may be added that our experience with the compound indicates an extreme degree of lability. To this, more than to differences in metabolic activity, we ascribe the deviations in results such as those seen between the two experiments using rabbit muscle (Experiments 1 and 2).

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Increase of Herbicidal Action of Concentrate 40 and Oil Emulsion by 2,4-D

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It is well known that 2,4-D is ineffective as a herbicide in the control of grasses. Nonselective herbicides such as Concentrate 40 and oil emulsions are often used to control grass weeds. Preliminary experiments conducted at the Federal Experiment Station in Puerto Rico have shown that Concentrate 40 + 2,4-D[1] and oil emulsion fortified with Santophen 20 + 2,4-D[2] both suppressed the population of "cohitre," or day flower (*Commelina longicaulis* Jacq.), and "bejuco de puerco" (*Ipomoea* spp.) (broadleaf plants easily eradicated with 2,4-D sprays) more than the same nonselective sprays without 2,4-D. The combination sprays also suppressed more weeds than 2,4-D alone. The results indicated that Concentrate 40 and oil emulsion fortified with Santophen 20, when used as a combination spray with 2,4-D, did not inhibit the lethal effects of 2,4-D and that it may be more effective than either nonselective herbicides when used alone on grass control.

In another experiment, an area completely covered with Bermuda grass (*Cynodon dactylon* (L.) Pers.), which is unaffected by 2,4-D and very resistant to arsenical, was divided into 10 equal plots. Five plots were treated with Concentrate 40 and 5 with 0.1% sodium salt of 2,4-D in Concentrate 40 at the rate of 175 gal/acre. Two uniform applications of both spray treatments were made at 4-week intervals, and the results recorded 20 days after the last application. The addition of 2,4-D to Concentrate 40 increased its herbicidal action against Bermuda grass by 50%. Plots sprayed with Concentrate 40 alone were completely covered with weeds, 60% Bermuda grass and 40% nutgrass (*Cyperus rotundus* (L.)). In plots sprayed with 0.10% 2,4-D in Concentrate 40 the area was covered with only 40% Bermuda grass and 5% nutgrass.

The results indicate that 2,4-D possibly activated the constituents in Concentrate 40, or vice versa, with a resulting synergistic reaction. The increased herbicidal effectiveness of the combination sprays may also be due to the injury caused by the constituents of Concentrate 40 (arsenic trioxide, Santobrite, and sodium chlorate), which enables the 2,4-D to enter the plant and exert its physiological effect.

[1] Consisting of 0.42% arsenic trioxide, 0.25% Santobrite (sodium pentachlorophenate), and 0.25% sodium chlorate plus 0.10% 2,4-D.

[2] Consisting of 10% diesel oil emulsion fortified with 0.7% Santophen 20 (pentachlorophenol).

Importance of the Methoxy Group in Antifibrillatory Compounds[1]

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It is of singular interest that all the potent compounds now in clinical use for their antifibrillatory activity possess a methoxy group. These include quinine (*7*), quinidine (*4*), α-fagarine (*3*), and recently atabrine (*5*). The methoxy group is present in a number of other drugs —notably, the antimalarial drugs, certain of the opiates, and colchicine. Of these later drugs, only to papaverine has antifibrillatory activity been attributed (*6*). In a preliminary study to clarify this point, measurements were made of the antifibrillatory activity of cinchonine and N-methyl-dibenzyl-amine with quinidine and α-fagarine as controls. Cinchonine was selected because it has the exact structure of quinidine minus the methoxy group. Similarly, N-methyl-dibenzyl-amine is closely related in structure (one less carbon in the amine chain) to α-fagarine, but lacks two methoxy groups and one dioxymethylenic group.[3] To aid in the comparison of these drugs the changes induced on blood pressure, pulse, electrocardiogram, and the acute fatal toxicity were also studied.

Cats anesthetized with Dial-urethane given intraperitoneally were used. The chest was opened and a pericardial cradle made. Electrodes were attached to the right auricle about 8 mm apart, always in the same location. The stimulating current was generated by a thyra-

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[3] N-methyl-dibenzyl-amine and α-fagarine were obtained from the Sterling-Winthrop Research Institute; quinidine and cinchonine, from the Fisher Scientific Company.

tron stimulator at a frequency of 600 impulses/min. The current necessary to just induce fibrillation was reduced to the minimum by connecting in series a suitable variable resistor in the external circuit. The resistance of the preparation was also measured. The maximum output of the stimulator was 250 v. Thus, by measuring the maximal resistance at which one could induce fibrillation, the threshold could be calculated in milliamperes. Most often this varied from 2 to 8 Ma, a value agreeing closely with that of Wegria and Nickerson, who used a somewhat similar method (*6*).

Blood pressure and pulse were recorded from the carotid artery. The EKGs were taken with a string galvanometer by use of needle electrodes inserted subcutaneously in the standard limb lead positions. Single doses of a particular drug were always given intravenously in 5 mg/kilo amounts. The typical experiment was run as follows: First, a series of at least three control thresholds were run, after which a 5-mg dose of the particular drug was given. Thresholds of fibrillation were taken immediately after (10 and 20 min). This procedure was repeated twice more. The changes in threshold reported in Table 1 are therefore the average result of 15 mg/kilo of drug in three divided doses at 20-min intervals. By giving 5-mg/kilo doses intravenously every minute and watching for cardiac arrest, the acute fatal toxicity was then obtained.

The results are summarized in Table 1. As was to be expected, quinidine proved to be an active antifibrillatory drug. By this method the average increase in fibrillation threshold was 69%. This was the figure obtained by averaging the results on 7 cats (9 separate thresholds on each cat over an hour period with a total dose of 15 mg/kilo). The maximum average effect of a 250% increase in threshold was observed after 10 mg/kilo had been given. There was a typical fall in blood pressure and a slowing of the pulse characteristic for this drug. The EKG changes consisted of widening the QRS complex and abnormalities of the S-T segment and T waves. There was frequently inversion of the QRS complex. The average acute fatal dose intravenously was 47 mg/kilo.

Surprisingly, cinchonine proved to be almost as active as quinidine. However, the maximum rise in threshold was only 101% and was obtained only after 15 mg/kilo had been given. Cinchonine was much less toxic than quinidine, the acute fatal dose averaging 80 mg/kilo. Moreover, the EKG changes observed in the case of quinidine were minimal in the case of cinchonine up to doses of 50 mg/kilo. A-fagarine was nearly 5 times more active than quinidine (308%). In every other respect it resembled the effects of quinidine on blood pressure, pulse, and EKG. Its acute fatal dose was greater (32.5 mg/kilo). N-methyl-dibenzyl-amine was not active in raising the fibrillation threshold. This is contrary to the results of De Espanes, *et al.* (*3*), who assigned considerable antifibrillatory activity to this drug. We believe our results to be correct, because this drug raised the blood pressure and increased the pulse rate. Moreover, no EKG changes were observed with any dose. Unfortunately, due to a shortage of the supply of the drug the acute fatal dose could not be obtained, but it was obviously much greater than 50 mg/kilo.

TABLE 1

Drug	No. of cats	Maximum avg. % inc. in threshold (Dose in par.)	Avg. % inc. in threshold over 1-hr period	Blood pressure	Pulse	EKG changes	Avg. acute intravenous toxicity (mg/kilo)
Quinidine	7	250 (10 mg)	69	Fall	Slowed	Marked	47
Cinchonine	7	101 (15 ")	67	Fall	Slowed	Slight	80
α-Fagarine	4	660 (5 ")	308	Fall	Slowed	Marked	32.5
N-Methyl-dibenzyl-amine .	4	36 (10 ")	12	Rise	Quickened	None	None with doses over 50 mg/kilo

Clearly indicated is the fact that omission of the methoxy and the dioxymethylenic groups from the structure of α-fagarine renders it impotent as an antifibrillatory drug and also removes its toxicity. Moreover, the compound that remains, N-methyl-dibenzyl-amine, is a pressor drug. This is not unexpected in view of its relationship to the sympathomimetic amines (*1*). On the other hand, cinchonine, which lacks the methoxy group, still retains considerable antifibrillatory activity, but its toxicity is much less and the typical EKG changes of quinidine are only slightly evident. Therefore, this observation indicates that the methoxy group is not necessary in the structure of an antifibrillatory compound. This is in accord with the observations of Dawes (*2*). However, our results permit the conclusion that the methoxy group and its related dioxymethylenic group are extremely important in the structure of the antifibrillatory drugs. If this observation is confirmed, it provides important leads not only in the search for the ideal antifibrillatory drug but in the study of the metabolism of muscle contraction.

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Inactivation of Nutrients by Heating With Glucose[1]

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Hill and Patton (*1*) found that the slight discoloration occurring during the autoclaving of media for the microbiological assay for L-tryptophan was caused by interaction with glucose, resulting in decreased growth of *Streptococcus faecalis* R. It was not known at that time whether the poorer growth resulted from destruction of nutrients or formation of growth inhibitors as products of the browning reaction. The work reported here indicates that the decreased growth is due to destruction of nutrients.

TABLE 1

LOSS OF L-TRYPTOPHAN DUE TO INTERACTION WITH GLUCOSE

Treatment	Browning (% T/610)	Hydroxymethyl-furfural (%)	Tryptophan loss (%)
Unheated	100	0	0
Heated	81.5	0.52	60
Heated at pH 10 ..	41.5	2.25	26

It is known that the browning reaction is promoted by alkalinity, and that hydroxymethylfurfural is one of the chief reaction products. Advantage was taken of these facts in the following tests: Aliquots of a solution containing known amounts of L-tryptophan and D-glucose were heated under suitable conditions to cause browning similar in appearance to that which occurred during autoclaving of media. The extent of browning was measured by determining the decrease in transmission at 610 mμ in a Coleman spectrophotometer. Hydroxymethylfurfural content, used as an indicator of the concentration of browning reaction products, was estimated from the absorption increase at 284 mμ in a Beckman DU spectrophotometer. The loss of L-tryptophan resulting from the browning reaction was determined by microbiological assay using the sucrose medium to prevent further browning loss. Aliquots buffered at pH 10 were prepared to obtain samples in which browning was only partially due to interaction with tryptophan. These mixtures produced more browning in less time. Growth of *Str. faecalis* R was measured turbidimetrically after 16 hrs by decrease in transmission at 610 mμ. At the dilutions used for assay, the browned samples were colorless at this wave length.

As shown in Table 1, better growth was obtained from the tryptophan-glucose sample heated at pH 10, in spite of the fact that more browning occurred and more hydroxymethylfurfural was formed. On the other hand, the solution containing only glucose and tryptophan, showing less browning and much less hydroxymethylfurfural formation although heated for a longer time, permitted poorer growth. These data indicate that decrease in growth was due not to formation of growth inhibitors as products of the browning reaction but to actual destruction of part of the tryptophan.

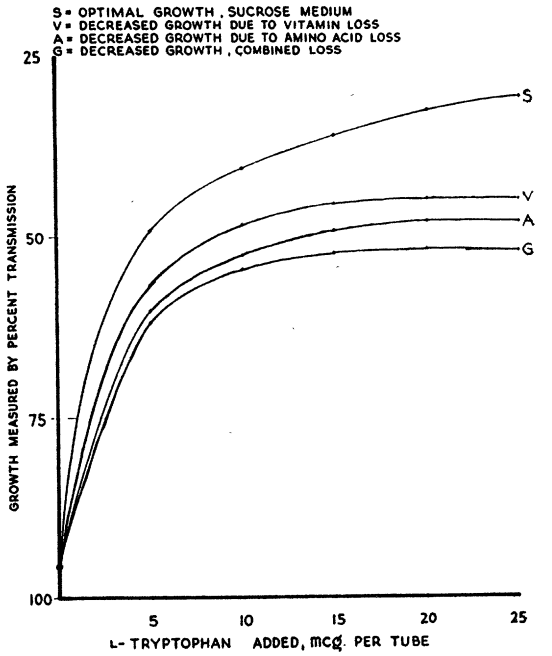

FIG. 1. Growth of *Str. faecalis* R in standard series for L-tryptophan assay, showing decreased growth due to autoclaving in presence of glucose.

It also appears that such destruction is not limited to tryptophan. Both L-lysine and DL-methionine, upon heating in the presence of glucose, underwent similar destruction, as determined by subsequent microbiological assay using synthetic amino acid media. Curve G (Fig. 1), which is a standard growth curve for L-tryptophan as obtained by the customary assay method using glucose in the medium, shows the total growth-decreasing effect of the browning reaction. Curve V resulted from the addition of a pure sterile solution of the amino acids to the autoclaved medium before inoculating, in an attempt to reveal the extent of vitamin destruction during autoclaving. Similarly, to produce curve A the vitamins were replaced after autoclaving, to show the extent of amino acid destruction. These curves indicate that nutrients in both the vitamin class and the amino acid class suffered

[1] Scientific Series No. 253, Colorado A & M College Experiment Station.