3 human subjects. Seventy to 83% of the administered substance was excreted unchanged, almost all within the first 6 hours after injection. The substance excreted was racemic: there was no preferential utilization or retention of acetyl-*l*-tryptophane.

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Effect of Quinacrine Hydrochloride (Atabrine) on Isolated Mammalian Heart.

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In laboratory animals (dog, cat, rabbit) the intravenous administration of atabrine causes a fall of blood pressure and, if the doses are large enough, disturbance of respiration.[1,2] As is the case with many substances, the toxicity is directly related to the rate of injection.[3] Studies on preparations of the isolated heart of the frog[4] as well as of mammals[1,5] suggest that atabrine has a negative inotropic action.

On the basis of the available clinical evidence[6-9] it appears possible that the negative inotropic action may play a rôle under certain clinical conditions when atabrine is injected intravenously in relatively large doses.

While the therapeutic levels, with the routine oral treatment now accepted, range between 25 and 75 μg per liter of plasma[10] and in total blood are of the order of 3 to 6 times these values;[9] in the experiments of Table VII in the paper of Shannon *et al.*, levels of the order of 1 mg per liter of whole blood and higher must have been present immediately after the rapid intravenous injection of the dose of 0.5 g.

Unna[2] ascribes the circulatory disturbance to action of atabrine upon the central nervous system. Before it is possible, however, to exclude the involvement of the heart in circulatory disturbances due to atabrine, it appears necessary to know at what atabrine blood level the heart begins to be affected. The following experiments on the heart-lung preparation of the dog were therefore undertaken to determine the minimum blood concentration of atabrine at which toxic effects are observed in the isolated mammalian heart.

It is fully appreciated that the atabrine concentration in whole blood does not represent the concentration in equilibrium with the body tissues because of the distribution of atabrine among the various components of the blood. It is our opinion, however, that our data are valid for the purpose of indicating the concentration range within which toxic effects upon the heart may be encountered, and that they apply to clinical

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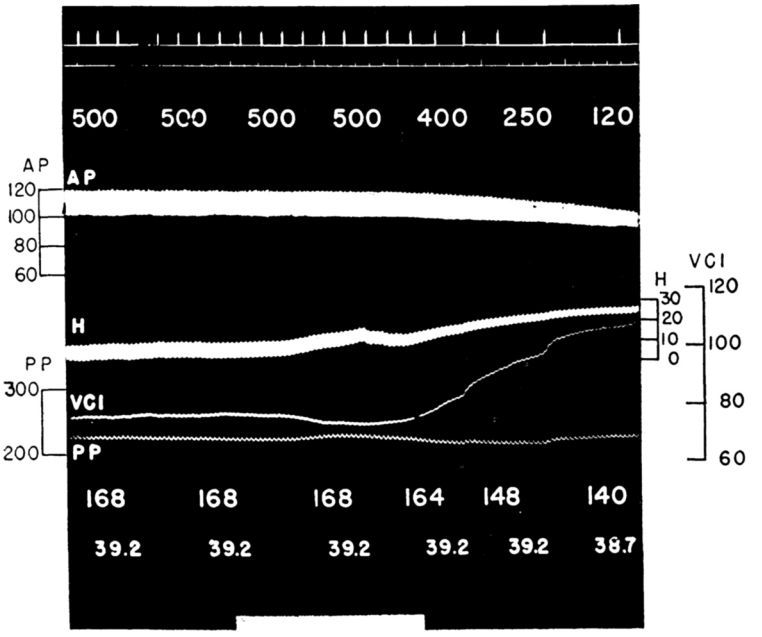

FIG. 1.

The effect of atabrine upon the isolated mammalian heart. Dog. Female. 8.2 kg. Heart-lung preparation. Arterial resistance, 78 mm Hg. Weight of ventricles, 49 g. Tracings indicate from above downward systemic output in 100 cc; time in 10-second intervals; arterial pressure (AP) in mm Hg; heart volume (H) in cc; right atrial pressure (VIC) measured from inferior vena cava in mm water. Pulmonary pressure (PP) in mm water recorded with a Bromoform manometer from a branch of the right pulmonary artery. The horizontal rows of figures indicate from above downward; systemic output in cc per minute; heart rate per minute; temperature of blood in degrees centigrade. During the period indicated by the signal 20 mg of atabrine were injected. The total blood volume was 830 cc.

conditions, as it is not unlikely that the human heart is more sensitive than the canine heart.[9]

Methods. Twelve heart-lung preparations were made according to Patterson and Starling.[11] The arrangement used was described in detail by Krayer and Mendez.[12] The dogs weighed between 9 and 12 kg. They were anesthetized with sodium pentobarbital 35 mg per kilo body weight injected intraperitoneally. Defibrinated blood was used. The total blood volume at the beginning of the experiments was between 600 and 900 cc. Arterial resistance was about 75-85 mm Hg to ensure a mean blood pressure between 100 and 120 mm Hg at a basal systemic output of 400-500 cc. Recordings were taken in all experiments of arterial pressure, right auricular pressure, pulmonary arterial pressure, heart rate, systemic output (= total output of left ventricle minus coronary flow). In some of the experiments heart volume, coronary sinus outflow, and electrocardiogram were followed. The atabrine was used in the form of the dihydrochloride. The doses and concentrations given refer to the salt.

To insure uniform distribution, fresh solutions of atabrine dihydrochloride were added to the blood before it entered the venous reservoir. Injections were made in single or divided doses, or by continuous infusion. Samples of blood were taken at intervals throughout the experiment and analyzed for atabrine, using the double extraction method of Brodie and Udenfriend.[13] Checks carried

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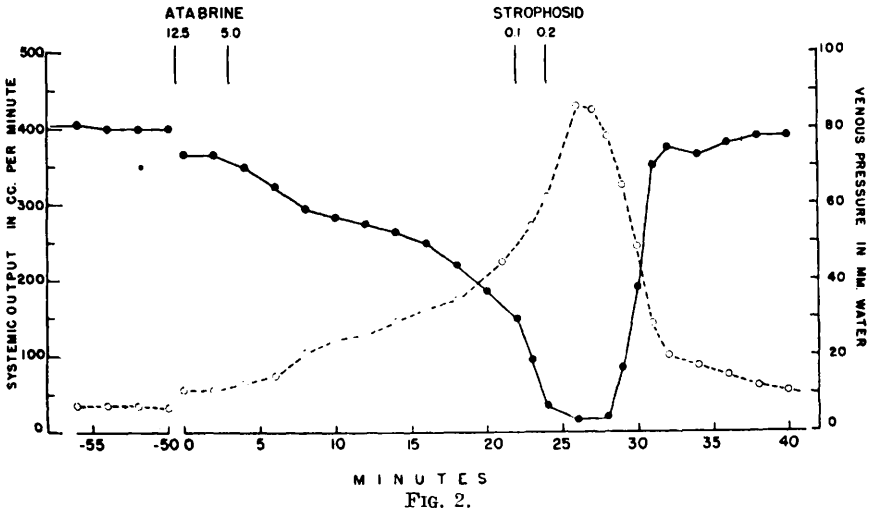

FIG. 2.

Effect of a cardiac glycoside on the myocardial failure caused by atabrine. Dog. Male. 9.7 kg. Heart-lung preparation. Arterial resistance, 78 mm Hg. Weight of ventricles, 75 g. ——●—— output in cc per minute, – – ○ – – right atrial pressure in mm water. The dosage figures of atabrine and strophosid indicate mg. (For further details see Exp. 6, Table I).

out with known amounts of atabrine added to defibrinated blood to determine the accuracy of the method gave recovery values of 90% or better.

Results. I. *The negative inotropic cardiac action of atabrine.* The characteristic effect of atabrine in the heart-lung preparation is an impairment of contractility leading to a decrease in output. This is illustrated by Fig. 1. In this experiment 20 mg of atabrine hydrochloride were administered by slow injection within 2 minutes. At the end of the injection the systemic output decreased to 80% of normal and within another 2 minutes fell to 24% of normal. At the same time there was a gradual increase in right atrial pressure and a marked increase in the volume of the heart. As there was no significant change in pulmonary pressure throughout the period of this change, the increase in volume of the heart must be ascribed largely to a negative inotropic action of atabrine. The decrease of the stroke volume of the heart can be seen from the width of the heart volume curve and is very marked in spite of the drop in heart rate to 83% of the normal rate.

As in all cases of heart failure occurring with this method of investigation, the increase in right atrial pressure was a good indicator of the onset, the development, and the intensity of the failure. The pulmonary arterial pressure changes were not consistent. In some experiments the pressure fell, in others it was not changed (as in Fig. 1), while in some it rose. Coronary sinus outflow measured in several experiments did not change significantly. The decrease in the systemic output of the heart, therefore, reflects the decrease in the output of the left ventricle and not a shunting of the blood through dilated coronary vessels as it may occur in spontaneous heart failure.

Bradycardia was not a constant finding. In 5 experiments the rate decreased by from 6 to 32 beats per minute. There was no correlation between degree of bradycardia and severity of the negative inotropic effect. In experiments without irregularities of rhythm the electrocardiogram did not reveal any characteristic differences from the normal records. In 4 experiments irregularities of rhythm occurred which seemed to be due to extrasystoles, but no electrocardiographic analysis was made in these experiments.

In no instance was the negative inotropic action or the disturbance of rhythm spontaneously reversed. In confirmation of ob-

TABLE I.
Concentration of Atabrine in the Blood of Heart-Lung Preparation of the Dog After Single and Divided Doses of Atabrine.

Exp. No.	Time, min.	Atabrine inj., mg	Atabrine conc., mg/L blood	Blood vol., cc	Wt of Heart	Remarks
2	0	15.0		785	80	
	2.5		5.9			Rapid rise of right atrial pressure, severe failure.
	11.0		4.1			
	17.4		3.1			
3	0	12.0		600	70	Marked irregularities of rhythm, rise of
	2.8		4.1			right atrial pressure.
4	0	10.0		780	65	
	2.5		1.4			Slow rise of right atrial pressure, gradual development of failure.
	9.0		1.0			
5	0	5.0		690	75	No effect.
	6.5	5.0		620		Slow rise of right atrial pressure,
	14.5		1.9			gradual development of failure.
	24.2	5.0		500		
	26.7		4.9			Severe failure.
6	0	5.0		875	75	
	11.2	5.0		860		No effect.
	15.0		1.4			" "
	22.5		1.0			" "
	27.2	2.5		810		
	30.7		2.0			Gradual rise of right atrial pressure.
	44.2	5.0		765		
	47.2		3.1			Severe failure.
	52.7		2.6			
	61.7		2.1			(See Fig. 2).
7	0	5.0		840	91	
	8.3		0.2			No effect.
	12.5	5.0		775		
	16.0		1.0			Slow rise of right atrial pressure, gradual development of failure.
	23.5		0.8			

servations of De Langen and Storm it was found that epinephrine hydrochloride readily restored an increased work capacity to the failing heart. The effect, however, was transient. Cardiac glycosides, on the other hand, had a long-lasting improving effect upon contractility. Ouabain and K-strophosid were each used in several experiments. Fig. 2 illustrates the effect of K-strophosid. The restoration of the work capacity of the heart is seen to be virtually complete. There was no significant change in heart rate in this experiment. Even if the heart failure occurred much more acutely than in the experiment of Fig. 2, the cardiac glycosides were able to effect a complete reversal.

II. *The minimal toxic blood concentration of atabrine.* Blood levels were determined in 7 of our experiments. The results are shown in Table I and Table II. The minimal toxic blood concentration is approximately 1 mg per liter. Concentrations below this were not found to have an effect; above 1.5 mg per liter negative inotropic effects were found to occur regularly. Large single doses caused severe toxic effects very quickly when injected rapidly, while the same dose given over a period of 2 minutes (see Fig. 1) led to negative inotropic effects which developed more gradually and as a rule were less severe. By slow administration relatively large amounts could be introduced without leading to toxic

TABLE II.

Concentration of Atabrine in the Blood of the Heart-Lung Preparation of the Dog During Constant Infusion. (Exp. 8). Dog, male, 9.5 kg. Arterial resistance, 70 mm Hg. Heart weight at end of experiment, 75 g. Infusion rate at beginning, 0.5 mg per minute, from minute 53 on, 1 mg per minute.

Time, min.	Concentration mg/L blood	Total blood vol., cc	Remarks
0	—	705	Infusion starts.
17	1.1	680	No effect.
28	1.2	650	" "
37	—		Right atrial pressure begins to rise.
40	1.7	620	Right atrial pressure continues to rise gradually.
50	2.0	570	Some decrease in competence.
53	—		Rate of infusion doubled.
58	4.1	510	Marked increase in right atrial pressure. Rapid development of myocardial failure.
62	6.1	470	

effects as in the experiment of Table II. In this experiment atabrine was administered during the first 50 minutes at the rate of 0.5 mg per minute. At 28 minutes when 14 mg had been injected, the blood level was 1.2 mg per liter and no negative inotropic effects were noticeable. These started at 37 minutes at a blood concentration between 1.2 and 1.7 mg per liter, which is in good agreement with the results presented in Table I.

III. *The disappearance of atabrine from the blood.* From experiment 2 of Table I it is evident that the atabrine concentration in blood decreased by 50% in 15 minutes. The trend of this decrease is obvious in several of the other experiments. Similarly, from the experiment of Table II it follows that atabrine is removed from the blood almost as rapidly as it is infused. After 50 minutes when 25 mg of atabrine had been infused at a rate of 0.5 mg per minute the concentration in the blood was 2.0 mg per liter. This means only 1.1 mg, or 4.5% of the amount administered, remained in the total blood volume of 550 cc. When the infusion rate was at this time increased to 1.0 mg per minute, a rapid rise in blood level from about 2 mg per liter to 6 mg per liter occurred within 10 minutes, corresponding to a retention in the blood of about 20% of the atabrine administered during this period.

Discussion. While Unna concluded from his experiments in intact cats that the toxic circulatory action is to be attributed to a central vasomotor depression occurring concomitant with respiratory stoppage, our experiments on the dog heart prove, in confirmation of observations of Hecht[1] and De Langen and Storm,[2] that atabrine has a negative inotropic action on the isolated heart. Furthermore, severe myocardial impairment can occur with no or only a slight decrease in rate and no marked changes in the electrocardiogram.

The minimal negative inotropic concentration of 1 mg per liter of blood admittedly is far above the therapeutic blood concentration required in human malaria. With appropriate doses given orally, levels of 1 mg per liter of blood are not reached. There can be no doubt, however, that intravenous injections rapidly performed may increase to dangerous levels the atabrine concentration reaching the coronary circulation, as evidenced by the figures of whole blood concentrations in the experiments of Shannon *et al.* in man quoted above.[9]

The experiment of Table II indicates the rate at which administered atabrine is removed from the blood, although in the heart-lung preparation the heart itself and the lungs are the only organs to take up the drug. The rapid rise of blood concentration when at minute 53 the rate of infusion is doubled suggests 2 probable explanations: either the tissues may have been nearly saturated by the infusion of approximately 25 mg and the rate of removal would have dropped off even with the low rate of administration; or heart and lungs remove atabrine from the blood at a limited rate and with an increase in rate

of administration above 0.5 mg per minute concentration in the blood rises steeply because the rate of removal does not correspondingly increase. This question needs further study.

A comparison of the results of Table I and Table II explains why injudiciously fast intravenous administration of atabrine may readily lead to toxic effects upon the heart muscle in the isolated heart, not encountered when even large amounts are given by slow intravenous administration, and entirely unlikely in the intact animal or man when appropriate therapeutic doses are given orally.

Summary. In the isolated heart of the dog in the form of the heart-lung preparation atabrine has a negative inotropic action which appears in some cases at concentrations of 1 mg per liter of blood and is always observed at concentrations of 1.5 mg per liter or above. As a result, the work capacity of the heart is impaired. Slowing of the heart rate sometimes occurs. Irregularities of cardiac rhythm are seen only with high and acutely toxic doses.

Injection of 10-15 mg of atabrine within 1 minute into a total blood volume of 500 to 900 cc leading abruptly to blood levels of several milligrams per liter of blood causes a severe heart failure, while the continuous infusion of the same amount at a rate of 0.5 mg per minute will barely reach the toxic concentration in the blood.

A severe myocardial failure of the isolated heart, once established, does not appear to be reversible. Epinephrine hydrochloride has a transient positive inotropic and chronotropic action, while the cardiac glycosides effect a prompt and long-lasting improvement of the work capacity of the failing heart in doses which do not change the heart rate and do not lead to irregularities of heart rhythm.

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Control of pH in Roller Tube Cultures.

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The purpose of these experiments was to determine in roller tube cultures the pH range of media which would support growth of fibroblasts. The study was undertaken as preliminary to the cultivation of bone *in vitro* under standardized conditions. It was prompted by the fact that the optimal reaction for the activity of alkaline bone phosphatase lies between pH 8 and pH 10.[1] Such values are distinctly beyond the range of pH considered desirable for tissue *in vitro*. In the case of chick tissue the accepted pH range is 7.2 to 7.8.

Various methods of controlling the pH range suggested themselves. The obvious was either to choose buffers operative in this alkaline range (8 to 10) or to use the ordinary solutions, such as Tyrodes, and control the amount of CO_2 in equilibration with the media. We chose the latter approach and devised an apparatus for continuous introduction of the gas.

Technic. The apparatus used to introduce gases into the roller tubes has been described.[2] The following gases were introduced into the tubes: air without CO_2; air; air with 1%, 2%, 4%, 8%, 16% and 32% CO_2. Air with no CO_2 was made by first drawing approximately 18 liters of air into a 5-gallon bottle and introducing a 10% NaOH wash bottle in the pipe line to the roller tubes. Air alone required no special treatment. All of the CO_2 mixtures were made with the aid of a Kipp generator. The gas displaced the proper amount of water and by siphoning

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