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THE MECHANISM OF DINITROPHENOL HEART FAILURE

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Hypoxaemia, resulting from increased tissue metabolism, is an important factor in dinitrophenol failure in the conventional heart-lung preparation. Improved oxygenation of the blood by a technique described in this paper prolongs the life of dinitrophenol-treated hearts. Dinitrophenol acts very rapidly; oxygen consumption and coronary flow increase in a few minutes and the increase is proportional to the dose. The increase in oxygen consumption diminishes with time. Dinitrophenol decreases the phosphocreatine content of the heart, even when there is no failure or hypoxia. There is no evidence that dinitrophenol failure can be due to a decrease of phosphocreatine or adenosine triphosphate content of the heart, although this is to be expected in view of the observed " uncoupling " action of dinitrophenol.

Results reported by Fawaz, Hawa, and Tutunji (1957) do not answer the following questions: (*a*) Are the low phosphocreatine values associated with dinitrophenol heart failure due to the direct " uncoupling " action of dinitrophenol or are they caused by hypoxaemia resulting from increased metabolism? (*b*) Is the decrease in phosphocreatine the cause of the failure? Furthermore, serious objections were raised by us against the measurement of coronary sinus flow by means of the Morawitz cannula technique. The importance of measuring coronary flow accurately cannot be over-emphasized, because a decrease in systemic output may simulate failure, when in reality a corresponding increase in coronary flow may be taking place.

METHODS

Coronary flow was measured by the Rodbard technique (1953) as adapted to the heart–lung preparation (Badeer, 1955). We used this standard procedure in one set of experiments, ventilating the lungs with pure oxygen and bubbling oxygen through the venous reservoir. In another set of experiments the oxygenation of the blood was improved by diverting the coronary venous blood to the venous reservoir for oxygenation. This modification is illustrated in Fig. 1. The heart–lung preparation was allowed to run for a short time with the venous inflow cannula inserted into the distal end of the left pulmonary artery. The venous inflow tube was then clamped and the peripheral resistance reduced. The proximal end of the right pulmonary artery was cannulated and the coronary venous blood, collected in a beaker, was returned to the venous reservoir. The distal end of the right pulmonary artery was connected to the venous reservoir. The venous inflow was then re-established and the peripheral resistance adjusted so that the blood pressure was maintained at 10 cm. Hg. The venous inflow level was kept at 30 to 35 cm. above the left auricle and this yielded a systemic output of 600 to 900 ml./min., an amount similar to that obtained with the conventional heart-lung prepara-

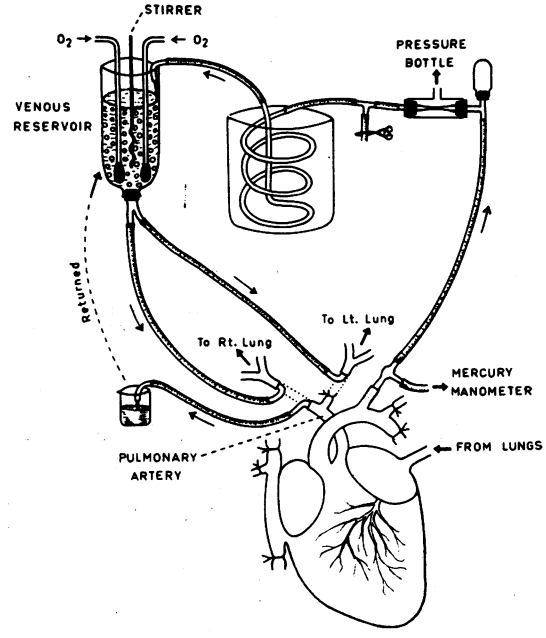

FIG. 1.—Modified heart-lung preparation to permit measurement of total coronary flow and myocardial oxygen consumption, and to improve arterial oxygen saturation in dinitrophenol experiments.

tion. The right heart was made to pump against a pressure of 16 cm. of water. This level was chosen because higher pressures resulted in failure of the right heart. Good arterial saturation was ensured by bubbling oxygen by means of two Pyrex glass dispersion tubes with fritted cylinders inserted at the bottom of a column of blood 7 cm. in diameter and 14 cm. in depth in the venous reservoir.

Dinitrophenol sodium was added slowly to the reservoir at 3 min. intervals in doses of 45 mg. If more than a total of 90 mg. was used, an interval of 10 min. was allowed to elapse before the third dose was given.

The phosphorus compounds were determined by the method of Fawaz *et al.* (1957). Adenosine triphosphate (ATP) was estimated chromatographically on several dinitrophenol-treated hearts and was found to be about 90% of the 7 min. hydrolysis value as reported in the tables.

Results

Experiments Utilizing the Rodbard Technique

Experiments Terminated Before Failure.—In this group, with a dose of 60 to 90 mg. of dinitrophenol, the experiment was terminated before any evidence of gradual or sudden failure developed. There was no decrease in total left ventricular output or of work performed after 40 min. There was an increase in coronary flow which was greater than that observed in control experiments of the same duration. The average oxygen content of coronary arterial blood at the end of the experiment was 13.1 vol.% as compared to 18.4 before dinitrophenol was added. The oxygen content of coronary venous blood did not change. The phosphocreatine values were about half the values obtained in similar control experiments and the labile nucleotide phosphorus did not change (Table I).

Experiments Terminated at the Onset of Sudden Failure.—The preparations survived from 10 to 41 min. after a dose of 90 mg. This confirms the observation of Gruhzit and Farah (1955) that there is great variation in the susceptibility of different hearts to dinitrophenol. There was again no decrease in output or performance of the heart before the onset of sudden failure. However, the average arterial oxygen saturation (8.5 vol.%) and the coronary venous oxygen saturation (3.1 vol.%) at the end of the experiment were significantly lower than the figures obtained in experiments terminated before failure. The phosphocreatine content was also lower, averaging about one-fourth the normal figures. The ATP values were insignificantly lower. Table I summarizes these results, and it seemed that, in this group of experi-

TABLE I

EFFECT OF DINITROPHENOL SODIUM ON THE DOG HEART-LUNG PREPARATION PERFORMED WITH THE RODBARD TECHNIQUE

Total Dose of Dinitrophenol Na	Duration of Expt. in Min.	Coronary Flow (ml./min.)		Total Left Vent. Output (ml./min.)		Change in Work Performed (%)	Change in O_2-Consumption (%)	O_2-content of Arterial Blood (Vol.%)		O_2-content of Coronary Venous Blood (Vol.%)		Phosphorus Compounds of Heart Muscle (% of Total Acid-soluble-P)		
		Begin.	End	Begin.	End			Begin.	End	Begin.	End	Phosphocreatine	Inorganic Phosphorus	Labile Nucleotide-P (2/3 of ATP)
Experiments terminated before sudden failure														
60	40	60	130	774	947	+23	+97	15·3	12·6	5·4	3·6	16·4	22·3	29·6
60	40	64	268	694	931	+39	+86	17·4	10·8	4·8	5·2	20·0	22·4	27·4
60	40	48	104	521	954	+98	+58	19·0	14·5	8·3	6·7	10·1	19·8	33·4
90	40	52	220	621	554	−14	+103	17·9	11·9	6·3	6·3	8·4	24·0	32·4
90	40	80	252	700	1,381	+117	+129	17·9	15·2	4·7	5·6	12·1	19·7	32·5
90	40	128	260	837	1,126	+37	+57	18·6	15·1	5·0	4·6	16·5	17·4	31·3
90	40	46	300	411	774	+93	+161	19·8	10·9	6·1	5·5	11·3	20·2	34·7
90	40	40	320	452	700	+52	+242	17·9	10·7	5·4	5·4	9·7	19·0	36·7
90	40	68	240	819	972	+20	+35	22·2	16·2	6·2	9·3	9·8	23·2	32·5
Average ±S.E.		65	233	659	926			18·4	13·1	5·8	5·8	12·7±0·9	20·9±0·7	32·3±0·9
Experiments terminated at onset of sudden failure														
90	28	138	308	1,110	1,131	+5	−69	18·8	4·5	7·2	2·9	1·0	31·2	30·4
90	20	96	288	826	917	+5	+50	21·6	9·3	5·6	1·4	1·4	30·8	29·3
90	15	132	292	505	441	−32	−47	20·5	6·2	5·6	2·7	5·4	35·8	25·2
90	24	120	480	456	840	+85	+121	16·4	6·4	8·0	1·8	8·9	24·2	32·0
90	15	84	464	668	1,230	+97	+218	16·4	8·1	9·0	3·8	12·5	25·2	28·8
90	10	92	420	605	860	+42	+268	19·7	12·5	9·9	4·5	15·9	25·6	29·0
90	41	68	224	315	350	+3	+136	20·0	11·4	5·3	0·9	2·0	32·1	27·2
90	38	76	440	638	1,384	+128	+88	17·9	12·3	7·1	8·8	10·3	19·6	30·8
90	33	76	412	636	1,029	+80	+164	18·1	6·2	9·9	2·0	0·8	34·7	23·3
90	11	48	300	605	750	+24	+263	17·6	8·2	6·6	1·8	1·2	38·0	28·2
Average ±S.E.		93	363	636	893			18·7	8·5	7·4	3·1	5·9±1·8	29·7±1·5	28·4±0·8

EFFECT OF DINITROPHENOL SODIUM ON THE DOG HEART-LUNG PREPARATION PERFORMED WITH THE MODIFIED TECHNIQUE

Total Dose of Dinitrophenol Na	Duration of Expt. (min.)	Coronary Flow (ml./min.)			Total Left Vent. Output (ml./min.)			Change in Work Performed (%)		Change in O_2-Consumption (%)		O_2-content of Arterial Blood (Vol.%)			O_2-content of Coronary Venous Blood (Vol.%)			Phosphorus Compounds of Heart Muscle (% of Total Acid-soluble-P)		
		Begin.	Middle	End	Begin.	Middle	End	Middle	End	Middle	End	Begin.	Middle	End	Begin.	Middle	End	Phospho-creatine	Inorganic-P	Labile Nucleotide-P (2/3 of ATP)
Short-term experiments terminated before sudden failure																				
90	23	23		192	709		727		−20		+373	18·7		16·8	6·2		9·8	16·4	19·5	33·3
90	28	52		252	681		421		−31		+419	15·6		14·9	4·6		5·2	20·0	19·7	31·4
135	30	58		264	520		494		+5		+206	17·8		16·1	7·0		8·5	11·5	24·1	31·6
135	29	41		366	705		631		−20		+298	14·5		9·0	4·4		4·5	5·5	25·8	33·6
135	31	38		276	678		556		−33		+198	21·7		16·8	6·6		10·6	10·2	19·7	34·6
135	24	72		440	739		724		−13		+240	16·0		14·8	8·8		10·7	10·3	25·2	30·4
135	26	50		424	715		826		+13		+439	22·2		18·7	12·6		12·6	12·2	23·0	30·0
Average ±S.E.		48		316	678		626					18·1		15·3	7·2		8·8	12·3±1·8	22·4±1·0	32·1±2·1
Long-term experiments terminated before sudden failure																				
90	87	52	280	300	657	764	534	+16	−23	+253	+221	19·0	15·4	13·6	5·9	6·8	6·3	16·2	20·5	31·2
90	159	40	110	116	788	601	320	−27	−65	+143	+56	19·7	17·9	17·2	7·6	7·2	10·7	20·5	18·2	36·2
90	152	52	286	252	549	495	493	−13	−13	+228	+66	17·1	11·1	9·9	8·9	6·2	7·1	9·9	24·4	30·4
90	120	94	224	196	1,267	966	636	−28	−54	+108	+77	13·1	12·6	12·6	5·9	6·3	6·5	13·0	18·8	34·6
180	94	30	390	300	717	621	438	−20	−44	+540	+239	18·6	9·4	13·1	7·4	4·2	9·3	14·7	22·4	27·6
180	116	30	296	276	777	734	472	−5	−45	+451	+266	20·4	18·7	20·4	6·8	11·1	15·0	7·9	22·5	32·2
180	83	44	300	200	996	533	328	−53	−60	+437	+169	18·3	15·3	16·1	3·6	3·7	7·4	12·6	22·2	30·8
Average ±S.E.		49	269	234	822	693	460					18·0	14·3	14·7	6·6	6·5	8·9	13·5±1·6	21·3±0·8	31·9±1·1
Experiments terminated at onset of sudden failure																				
90	96	68	232	240	675	831	530	+27	−29	+113	−3	16·6	13·6	10·2	5·7	6·8	7·2	11·4	23·2	33·4
90	134	38		168	570		257		−59		−19	20·0		18·4	5·7		15·8	10·8	20·0	34·7
90	152	32	194	116	539	381	200	−34	−70	+253	+20	19·1	11·9	19·1	7·6	5·2	15·3	13·8	23·1	31·7
90	115	56	166	200	1,298	1,143	292	−17	−85	+67	−10	22·4	20·6	20·6	7·3	12·1	16·8	4·1	37·0	23·6
135	106	42	312	216	646	655	478	0	−32	+232	+113	20·2	13·5	9·0	7·2	7·7	3·6	4·7	21·6	34·8
180	117	40	380	120	668	586	192	−18	−75	+415	+48	22·8	15·2	20·1	9·6	8·0	13·6	13·9	19·2	31·5
180	92	44	340	192	1,368	890	319	−44	−82	+331	+156	18·0	13·7	16·6	4·7	6·3	8·8	3·3	26·2	30·6
Average ±S.E.		46	271	179	823	748	324					19·9	14·8	16·3	6·8	7·7	11·4	8·9±1·8	24·3±2·3	31·4±1·4

TABLE III

CONTROL EXPERIMENTS

The first three experiments were performed by the Rodbard technique, the rest by the modified technique. *= Spontaneous failure. †= Barbiturate failure.

Duration of Expt. (min.)	Coronary Flow (ml./min.)			Total Left Vent. Output (ml./min.)			Change in Work Performed (%)		Change in O_2-consumption (%)		O_2-content of Arterial Blood (Vol.%)			O_2-content of Coronary Venous Blood (Vol.%)			Phosphorus Compounds of Heart Muscle (% of Total Acid-soluble-P)		
	Begin.	After 60 min.	End	Begin.	After 60 min.	End	After 60 min.	End	After 60 min.	End	Begin.	After 60 min.	End	Begin.	After 60 min.	End	Phospho-creatine	Inorganic-P	Labile Nucleotide-P (2/3 of ATP)
40	132		132	841		829		+1		0	19·5		19·5	5·1		5·1	22·5	17·4	32·0
40	92		196	941		1,080		+11		−4	17·7		16·9	8·2		12·6	23·1	15·7	31·2
40	140		152	634		733		+18		+43	15·7		17·5	8·7		8·3	26·8	15·5	26·1
122	24	50	66	266	499	409	+100	+62	+28	+11	18·2	18·1	18·2	6·8	11·1	13·6	18·8	19·9	27·8
122	36	74	202	784	801	762	+3	−2	−8	+1	22·6	23·0	21·8	8·3	16·6	19·3	27·7	12·0	26·4
120	42	63	208	886	1,173	969	+37	+9	+5	+65	20·8	20·0	21·1	6·5	10·0	16·3	24·4	15·3	30·4
121	40	56	72	908	1,226	1,243	+45	+38	+38	+24	18·0	18·0	20·4	5·4	5·6	11·7	22·5	18·5	32·8
44	30		26	261		77		−78		−43	21·6		20·8	5·4		9·9	29·4	11·8	30·1*
120	30		142	849		288		−95		−6	21·7		17·4	8·1		14·7	20·4	13·8	33·6†
110	42		114	562		211		−66		−13	19·0		17·0	9·9		14·1	22·9	12·4	32·0†
Average ±S.E.	61	61	131	693	925	660					19·5	19·8	19·1	7·2	10·8	12·6	23·9±1·0	15·2±0·9	30·2±0·8

ments, the heart failure was attributable to arterial hypoxaemia, probably due to failure of the lung to do its share in the oxygenation of coronary blood.

Experiments with the Modified Rodbard Technique

Short-term Experiments Terminated Before Failure.—The dose of dinitrophenol was increased to 90 to 135 mg. There was little change in total left ventricular output or work performed due to the drug, but there was a significant increase in oxygen consumption (200 to 400%). The arterial oxygen saturation at the end of the experiment was satisfactory and the oxygen saturation of coronary venous blood was high. The phosphocreatine values were about 50% of normal and there was no change in ATP (Table II).

Long-term Experiments Terminated Before Failure.—These experiments were allowed to run for a period of 83 to 160 min. with doses of 90 to 180 mg. of dinitrophenol. The increase in oxygen consumption after 30 to 60 min. was greater than at the end. There was also a decrease in total left ventricular output towards the end of the experiment, part of which may have been due to spontaneous failure. Here again arterial and venous oxygenation were satisfactory. Phosphocreatine values were about 50% of normal and there was no change in ATP (Table II).

Experiments Terminated at the Onset of Failure.—The dose of dinitrophenol was 90 to 180 mg. and the survival period was 92 to 152 min. These results, given in Table II, demonstrate again the effect of proper oxygenation in prolonging the survival period of heart-lung preparations treated with dinitrophenol. The oxygen consumption at the middle of the experiment was greater than at the end, and there was a decrease in total output and work performed towards the end. Arterial and venous oxygen saturations were very satisfactory. The phosphocreatine values were slightly less than half the normal.

Control Experiments

These were carried out on heart-lung preparations without dinitrophenol, and were allowed to run for 40 to 120 min. Three experiments were performed with the Rodbard technique, the rest with the modified procedure (Table III). One heart failed spontaneously, two were made to fail by the addition of pentobarbitone. There was a general increase in coronary flow and a corresponding increase in the oxygen saturation of coronary venous blood (average 12.6 vol.%). The phosphocreatine values were consistently high, averaging

24% of the total acid-soluble phosphorus. The changes in oxygen consumption and work performed as the experiment proceeded were not significant.

Discussion

One important fact which emerged from the foregoing experiments is that hypoxaemia, due to increased tissue metabolism, is an important factor in dinitrophenol failure in the conventional heart-lung preparation. Improved oxygenation of the blood resulted in prolongation of the life of the preparation. To what extent hypoxaemia has played a role in results of experiments published by others is difficult to say, but neither Rothlin, Taeschler and Cerletti (1955) nor Gruhzit and Farah (1955) mentioned observing hypoxaemia. Yet it is common knowledge that the oxygen saturation of the blood in an ordinary heart-lung preparation without dinitrophenol often markedly improves when the lungs are ventilated with oxygen instead of air.

It is surprising that the lungs in a heart-lung preparation are not able to oxygenate blood serving only these two organs, when *in situ* they serve the needs of the whole body. Could this " unphysiological " behaviour of the isolated lungs be due to the fact that the lungs *in situ* receive their nutrition through the bronchial arteries ?

Dinitrophenol acts very rapidly in increasing oxygen consumption and coronary flow. These effects are visible a few minutes after a dose of 45 mg. of the drug is added. The increase in the coronary flow and the corresponding decrease in systemic output may be so great as to simulate heart failure if no accurate method is available for measuring coronary flow. This is particularly true of short-term experiments. In general, the increase in oxygen consumption is proportional to the dose of dinitrophenol ; in long-term experiments the effect fades away towards the end, even if a correction is made for the decrease in work performed. We have no explanation for this finding.

Dinitrophenol decreases the phosphocreatine content of cardiac muscle in the heart-lung preparation. There is a 50% decrease even when the factor of hypoxaemia is eliminated and no signs of failure are present. Yet the conclusion cannot be drawn that dinitrophenol failure is due to its action of uncoupling phosphorylation and oxidation with the resulting unavailability of high-energy phosphate bonds. In the short-term experiments with proper oxygenation, terminated before failure, dinitrophenol exerted its maximum activity, increasing metabolism 2 to 4 times. This is clear evidence that the drug had diffused into the cells. The doses given, although in excess of those used in homogenates and isolated enzyme systems to uncouple phosphorylation and oxidation, did not produce failure at the height of their metabolic action. Furthermore, in the long-term experiments with bigger doses and proper oxygenation, terminated before sudden failure, where there was a gradual decrease in output and work performed towards the end, the phosphocreatine values were not lower than in the previous short-term experiments. Even in the experiments with proper oxygenation terminated during heart failure, the phosphocreatine values were not significantly lower than in the experiments terminated before sudden failure. It is therefore not possible to correlate dinitrophenol failure with a decrease in phosphocreatine content. What has been said of phosphocreatine applies also to adenosine triphosphate, which does not change after giving dinitrophenol. The results of these experiments are similar to those obtained with fluoroacetate on the heart-lung preparation (Fawaz, 1956). Fluoroacetate is also a substance which blocks the Krebs cycle at the citrate stage and would be expected to produce failure due to lack of availability of high-energy phosphate bonds. We found that fluoroacetate produced failure to a degree which was proportional to the dose of the drug. Citrate accumulation in the heart was also proportional to the dose of fluoroacetate ; phosphocreatine decreased, but there was little correlation between the degree of failure and the decrease in phosphocreatine. Here again, ATP did not change during failure.

To summarize, heart failure produced by hypoxaemia, ischaemia due to ligation of the coronary arteries, dinitrophenol with or without tissue anoxia, or by fluoroacetate is associated with a significant decrease in phosphocreatine but no change in the ATP content of the heart. Phosphocreatine breakdown may or may not have the function of replenishing the ATP reserves, but it is difficult to visualize why the heart should not continue its action if, as in all these cited cases of failure, its ATP stores are still intact. It is thus difficult to reconcile these findings with the accepted view that ATP plays the dominant role in supplying energy for cardiac muscle contraction, and one must be careful not to assume that results obtained *in vitro* with simplified systems should necessarily apply *in vivo*.

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