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UTILIZATION OF BLOOD SUGAR AND FORMATION OF LACTIC ACID BY THE LUNGS.

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Investigation of the changes brought about in the blood as a result of its passage through the coronary vessels of the heart [Evans *et al.* 1933; Rühl and Rolshoven, 1933; Rolshoven, 1933], has confirmed the work of McGinty [1931] and McGinty and Miller [1933], who found that the heart removes from the blood much more of lactic acid than of glucose.

It had been previously shown by Anrep and Cannan [1923] that on ventilation of the heart-lung preparation with air the lactic acid content of the blood in the venous reservoir often increased, whereas when carbon dioxide-oxygen mixtures were substituted for air, the lactic acid rose more slowly or even fell. A likely explanation of this phenomenon seemed to be [Evans *et al.* 1933] that the lactic acid content of the blood represented a balance between the rates of formation of lactate from blood sugar by glycolysis in the blood stream, and of its continual removal from the blood by the heart. A similar equilibrium is in all probability accountable for the analogous behaviour of the perfused limb-lung preparation [Eggleton and Evans, 1930].

In attempting to explain the phenomenon of the rise and fall of the blood lactate in the heart-lung preparation, it was provisionally assumed [by Evans *et al.* 1933] that lactic acid was formed in the blood from glucose at the same rate as in the blood *in vitro*. Although the lungs had been shown [Eggleton and Evans, 1930] not to remove any lactic acid from the circulating blood, the question of whether they added any to it was shelved for the time, since it seemed that glycolysis in the blood itself might account for all the lactate that found its way into the blood in those experiments. As in all cases the margin was small, however, while in some the heart seemed likely to be using lactate more quickly

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than the blood could form it, we undertook experiments in order to find whether the lung actually contributed lactate to the blood. They showed that it did so.

Some previous investigators had found lung tissue to cause glycolysis [Sluiter, 1924; Binet and Klukowski, 1933]; others failed to find it [Eppinger and Wagner, 1920; Laser, 1932].

Methods.

Dogs were generally used, but the lungs of the cat and sheep have also been tried.

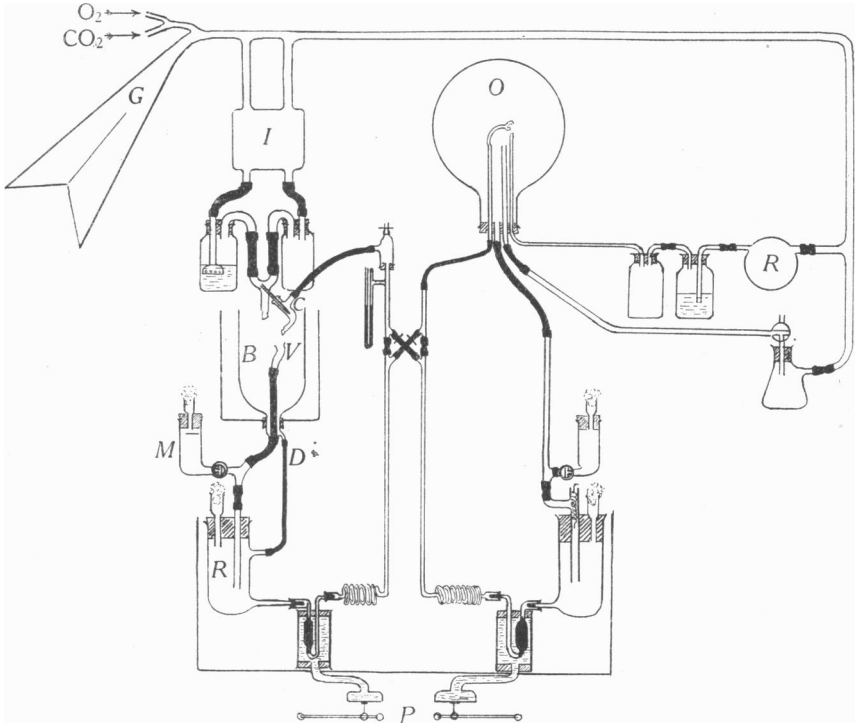

Fig. 1. The perfusion apparatus, with oxygenator control and gas circuits.

The most useful apparatus and procedure were as follows (Fig. 1): the blood, or other fluid, at 38° C., was passed by a Dale-Schuster perfusion pump (*P*) from a graduated reservoir (*R*) through a cannula (*C*) in the pulmonary artery into the exsected lungs; a cannula (*V*) tied into the left auricle returned the pulmonary vein blood to the reservoir, and the rate of this flow could be measured with the aid of a stop-watch by diversion into a calibrated receiver (*M*). Free-draining blood from

bronchial vessels was collected by suspending the lungs in a warmed cylindrical receiver (*B*) from the sump of which a drain (*D*) led back to the reservoir. The lungs were ventilated by an "Ideal" respiration pump (*I*), the air from which was moistened by being bubbled through water at 37° C.: in order further to reduce evaporation the lungs were enveloped in a cape made of cellophane or moist peritoneal membrane, over which some gauze, moistened with salt solution, was placed. As a control for determining the rate of glycolysis in the blood alone, in the earlier experiments a like quantity of blood was simultaneously circulated at a similar rate through an oxygenator (*O*) [Euler and Heymans, 1932] in which oxygenation was effected in a spray blown with air or other gas mixture. Mixtures of gases of approximately the required composition were made by the use of metering valves [Williams, 1933] on the cylinders from which the storage bag was frequently replenished. The gas mixture used was in all cases the same as for ventilation of the lungs, both being fed from the same storage bag (*G*) and circulated by a rotary pump (*R*). The circulations of blood through lungs or oxygenator could at any time be changed over, so that the blood which had been passing though the lungs could be made to pass through the oxygenator and *vice versa*. Difficulties with frothing were not quite overcome with this oxygenator when using air or mixtures not rich in oxygen, and ultimately the apparatus was simplified by replacing the parallel control with the oxygenator by the use of a small sample of the blood exposed at 38° C. to a gas mixture identical with that used to ventilate the lungs. It was found to be almost immaterial whether this sample of blood was kept in constant motion or only occasionally disturbed (Fig. 2). This is an important point, since in some of our experiments the blood used for the control determinations was much less disturbed than that passing through the lungs; in order to reduce any error due to this cause we always thoroughly mixed the stationary control blood at more frequent intervals than in the experiments of Fig. 2, but in the majority of the experiments it was kept in constant motion, either by being circulated through the oxygenator or by being rotated in a saturator containing a gas of the same composition as that used for ventilating the lungs. Usually the blood was defibrinated and strained through fine muslin, but in some experiments anti-coagulants were used instead. Microscopic examination of the defibrinated blood before and after prolonged perfusion showed no fibrin threads, and apparently normal erythrocytes. The operations were carried out with all convenient precautions of cleanliness and in some cases with rigid asepsis.

The procedure finally adopted was to furnish the dry and invariably heat-sterilized perfusion apparatus with a measured amount of blood from a first dog: the difference between the measured volume and the reading on the graduated reservoir gave the dead space of tubing, etc. The lungs from a second dog were then removed and cannulated, rapidly washed through with defibrinated (or incoagulable) blood delivered by gravity from a funnel, weighed and connected to the proper perfusion apparatus, and artificial respiration and blood circulation

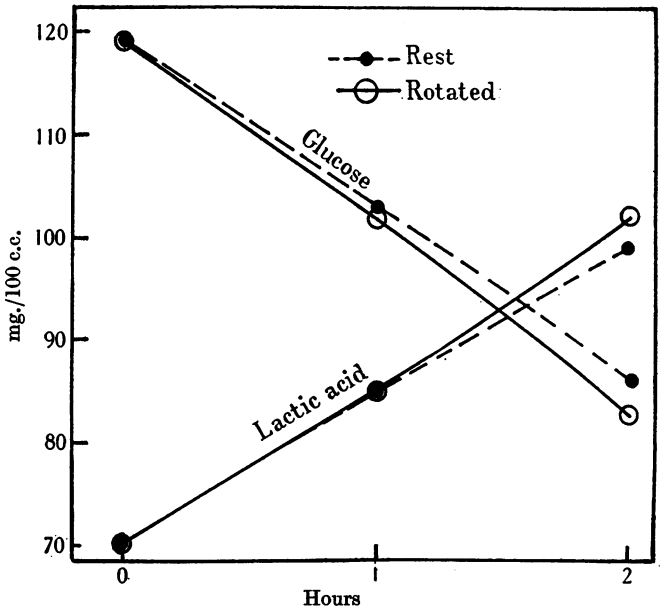

Fig. 2. Blood glycolysis *in vitro*. Effect of constant rotation. Mean curve for three experiments. (In two of these the effect of rotation was negligible.)

begun. After 1 or 2 min. the perfusion pump was stopped for about 20 sec. and the reservoir level read; the reduction in level showed the amount of blood held at about zero pressure in the lung vessels. It was often surprisingly large, and when the pump was restarted became larger still (usually by about 50 c.c. for a lung of about 100 g. weight). The rate of blood flow used was, if possible, adjusted so as to give pulmonary artery pressures not greater than 60 mm. Hg: with flows up to 500 c.c. per minute for lungs of about 100 g. the pressure was usually about 30 mm. Hg. The lungs were again weighed at the close of the experiment.

Samples of blood from the reservoir or venous return tube were taken at convenient intervals for determination of true glucose and lactic acid, in duplicate, and also in many cases of percentage hæmolysis, hæmoglobin content [Sahli], oxygen content and percentage saturation [Haldane], carbon dioxide content [van Slyke], hydrogen ion concentration (dialysis). Blood sugar was estimated by Shaffer and Somogyi's method 50 [1933], following deproteinization by Somogyi's cold zinc hydroxide [1930] or copper method [1931]. Lactic acid by deproteinization with trichloracetic acid and estimation by the method of Friedemann, Cotonio and Shaffer [1927].

Results.

Glycolysis in vitro.

The graphs showing the rate of usage of glucose and of accumulation of lactic acid for blood *in vitro* are nearly rectilinear for the first hour or two, and the rate of change as an average of twenty-two experiments for blood in equilibrium with air (Table I) showed a loss of glucose of 16·2 mg./100 c.c./hour (max. 23, min. 8), and a gain of lactic acid of 15·5 mg./100 c.c./hour (max. 22·2, min. 8), the average yield of lactic acid from glucose being thus about 96 p.c. We suspect that small amounts of substances other than lactic acid and/or glucose were estimated by the methods we used, however, and among other reasons because we have not infrequently found that slightly more lactic acid appeared than corresponded with the apparent glucose lost. In fifteen of these experiments samples of the same bloods were also equilibrated with mixtures of air (or oxygen) and carbon dioxide, containing on an average 7·15 p.c. CO_2 and gave average figures for glucose of 9·93 mg./100 c.c./hour

Table I. Glycolysis *in vitro*.

		Change, mg./100 c.c./hour		
No. of exps.	Gas phase	Glucose usage	Lactic acid formation	Lactic acid yield as p.c. of glucose
22	Air	16·2 { max. 23 / min. 8	15·5 { max. 22 / min. 8	96
15	7·15 p.c. CO_2 (av.) in air or O_2	9·93 { max. 16 / min. 5	9·90 { max. 18 / min. 6·3	99·7

(max. 16, min. 5) and for lactic acid of 9·90 mg./100 c.c./hour (max. 18, min. 6·3), with a yield of nearly 100 p.c. lactic acid from the glucose. The close correspondence between the glucose lost and the lactic acid gained

by the blood undergoing glycolysis *in vitro* is shown by the fact that the sums of the glucose and lactic acid present in the blood at beginning and end are nearly identical when a sufficient number of experiments are averaged together; in individual experiments, however, the sum at the close of the period of an hour may be greater or less than at the beginning. Usually it is less, and obviously on the average by an amount of about 0·7 mg./100 c.c./hour for blood in air. But in a large number of cases the deficit is above this average and sometimes reaches 3–5 mg./100 c.c./hour. We may suppose it to be due to loss by oxidation of either or both substances, though there are other possibilities. On the other hand, in a few cases the sum increases with glycolysis, which may be due to the formation of some other substances estimable as lactic acid, or to liberation of sugar from ester combination, etc. It is sufficient to note, however, that on the average the discrepancy is so small as to lie within the experimental error. The rate of glycolysis seems to be the same in blood rendered incoagulable by the addition of heparin or of chlorazol fast pink BKS [Huggett and Rowe, 1933] as it is in defibrinated blood.

Changes in blood during perfusion through lungs.

Although some of the experiments have been prolonged up to 5 or 6 hours, we consider that, owing to the ultimate occurrence, even in the best experiments, of some amount of lung œdema, and for other reasons which will be shown, conclusions drawn from results obtained more than 2 or 3 hours after the removal of the lungs from the body may be of doubtful significance. Whether of long or short duration, however, the experiments not complicated by copious œdema have, with one single exception out of a total of thirty experiments, all shown that the rates of lactic acid increase in and glucose usage from the blood are obviously accelerated by passing it through the lungs. The results of a typical experiment are shown in Fig. 3.

The figure shows a rapid fall in blood sugar and rise in blood lactate, and indicates that the rate of glycolysis is augmented from 15·7 mg./100 c.c./hour *in vitro* to 40 mg./100 c.c./hour in the lung circuit, while the lactate formation rises from 14·5 to 32 mg./100 c.c./hour. The graph is not quite linear, but shows a reduction in the rate of change as the perfusion goes on. The reduction in rate is actually greater than would appear from this method of presentation, where it is to some extent offset by the fact that the total volume of blood upon which the lung is acting is being continually reduced. The rate of change in fifteen similar experiments is given in Table II, from which it is seen that in the

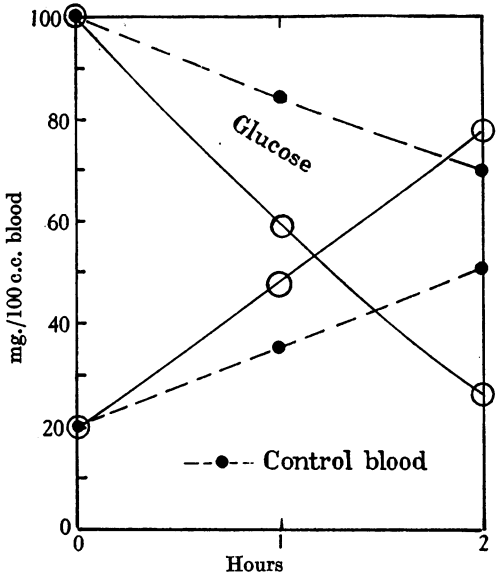

Fig. 3. Exp. 364. Glycolysis in pulmonary blood compared with control. Air ventilation.

TABLE II. Glycolysis in pulmonary blood and control. Air ventilation.

	Lung circuit							
	First period*			Second period*			Control	
		Mg.100 c.c./hour			Mg./100 c.c./hour		mg./100 c.c./hour	
Exp.	Blood vol. c.c.	Glucose loss	Lactic acid gain	Blood vol. c.c.	Glucose loss	Lactic acid gain	Glucose loss	Lactic acid gain
361	300	39	28	300	30	27	12·8	10·4
362	330	51	16	300	39	45	8·6	—
364	360	40	32	300	29	27	15·7	14·5
365	450	30	25	400	29	33	17·0	18·0
366	300	33	27	—	—	—	14·0	16·0
367	320	36	27	290	36	22	—	—
370	350	34	23	325	36	16	10·6	11·4
371	380	53	45	350	43	43	13·4	15·3
373	290	44	28	270	30	30	13·4	12·0
376	300	42	20	290	36	28	16·8	18·0
377	340	56	22	—	—	—	16·0	10·0
378	270	42	34	250	37	35	16·0	14·6
382	520	36	26	480	28	28	20·0	18·1
389	470	37	26	450	36	25	17·3	22·2
397	440	42	33	—	—	—	22·0	20·0
Average	362	41	27·5	333	33	30	15·2	15·4

By lungs alone, average, 1st period = 93·5 mg. glucose and 43·5 mg. lactic acid.
By lungs alone, average, 2nd period = 59·2 mg. glucose and 48·6 mg. lactic acid.
Per 100 g. lung/hour: 1st period = 112·5 mg. glucose and 52·5 mg. lactic acid.
2nd period = 71·3 mg. glucose and 58·5 mg. lactic acid.

* Usually of 1 hour.

first hour the blood loses on the average 41 mg. of glucose per 100 c.c. and gains 27·5 mg. lactic acid, or, deducting the change in the control blood, that the lungs use 93·5 mg. glucose and form 43·8 mg. lactic acid per hour, while during the second hour the change is 33 and 30 mg./100 c.c./hour (or total usage and formation of 59·2 and 48·6 mg.) respectively.

The average weight of the lungs was 83 g. and that of the heart 81 g., from which the change per 100 g. lung per hour is, for first hour, 112·5 mg. glucose and 52·5 mg. lactic acid, and for the second hour, 71·3 mg. glucose and 58·5 mg. lactic acid. Fig. 4 shows the mean graph of the fifteen experiments.

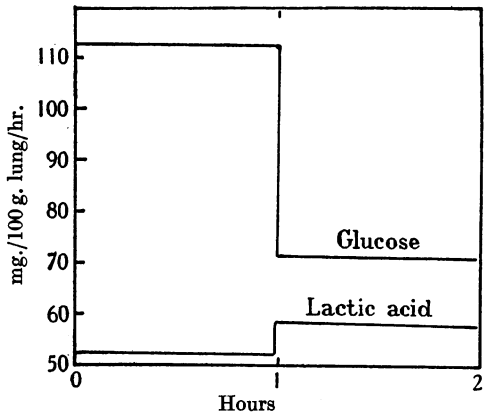

Fig. 4. Rates of glucose usage and lactic acid formation, mg./100 g. lung/hour, after deduction of change in control blood. From mean data of Table II.

There is at present no satisfactory explanation of the progressive decline in the rate of glycolysis. The figures for glucose usage for the first hour are somewhat higher than the values given by previous investigators, and this is no doubt in part due to the fact that it has often been the practice to consider longer periods. Thus Patterson and Starling [1913], although ignoring glycolytic changes in blood, obtained about 84 mg./100 g. (of accompanying heart)/hour, while Cruickshank and Startup [1930], who allowed for glycolysis, obtained 75 mg./100 g. heart/hour; Hemingway and Phelps [1934], who disregarded blood glycolysis but used brief perfusions and attempted to allow for glycogen change in the lung itself, found 84 mg./100 g. lung/hour.

The new feature of our experiments is that they show that the glucose disappearing from the blood is not all oxidized in the lungs, but that much of it is converted into lactic acid. The fraction so converted is less than that in the blood itself, and appears to be about 46 p.c. during

the first hour and 82 p.c. during the second hour. Ventilation with nitrogen containing 7 p.c. CO_2 and 1·6 p.c. oxygen, though it lowered the oxygen saturation of the blood from 100 to 26 p.c., did not increase the yield of lactic acid; no experiments under completely anaerobic conditions were performed.

We have so far not attempted to find to what extent the glycogen content of the lung itself is concerned in these exchanges, but it is possible that storage may have happened during the first hour of perfusion, since the blood sugar was usually high to begin with, and this, according to Cruickshank and Startup [1930], would favour glycogen formation in the lungs, which, moreover, the experiment quoted by Hemingway and Phelps [1934] would substantiate. If this is so it might account for the increasing yield of lactic acid as the perfusion proceeds.

When all the glucose has been removed from the blood, however, the lactic acid ceases to increase, and usually shows a slight fall, as the following experiments (Table III) illustrate:

TABLE III. Aglycæmic perfusion.

	Exp. 367		Exp. 371		Exp. 375	
Hour	Blood glucose mg./100 c.c.	Blood lactate mg./100 c.c.	Blood glucose mg./100 c.c.	Blood lactate mg./100 c.c.	Blood glucose mg./100 c.c.	Blood lactate mg./100 c.c.
0	116	62	155	30	106	32
3	22	128	24	133	8	96
4	0	144	0	142	2	102
5	0	140	0	134	0	99
6	0	133	—	—	—	—
Average hourly fall in sum of glucose + lactate		7·5 mg.	—	10 mg.	—	6 mg.
Average fall of lactate after disappearance of sugar		5·5 mg.	—	8 mg.	—	—

The fall in lactate occurs at a rate which is rather less than that previously shown by the rate of diminution in the sum of sugar and lactate; this latter may be taken to represent the carbohydrate stored or eventually oxidized, either directly or after being first converted into lactic acid. These data may be compared with those for the oxygen usage of the lungs obtained by Evans and Starling [1913]; their figure was 1 c.c./g. heart/hour. Since 1 c.c. oxygen = 1·34 mg. glucose or lactic acid, the amount of oxygen used, directly or indirectly per hour in combustion of glucose, in our experiments of Table II would be (93·5 − 43·8)/1·34 = 37 c.c., which, for an average heart weight of 81 g., would be only 0·46 c.c. O_2/g. heart/hour. Apparently, therefore, the lung tissue does not draw its entire combustible material from blood sugar or lactate.

Certain considerations had to be weighed, however, before it could be concluded that lactic acid was actually being formed from blood sugar in the lungs more rapidly than would occur spontaneously in the blood alone, and before we could conclude that such accelerated glycolysis was a normal occurrence.

Thus a fraction of the blood in the pulmonary circuit was in rapid movement and could not without investigation be compared with control blood which sometimes remained relatively at rest. That continuous rotation of the blood in a saturator did not result in an appreciably greater rate of glycolysis than occurred in blood only periodically stirred round has been already mentioned, but in order to have more comparable movements and to expose both bloods to similar gas mixtures the oxygenator circuit control was employed in some experiments. This, however, introduced a fresh source of error, in that considerable hæmolysis occurred in the blood of the oxygenator circuit. Whereas the hæmolysis in the fresh defibrinated blood was of the order of 0·5–1 p.c. rising usually to 1–2 p.c. after several hours of lung perfusion, the hæmolysis in the oxygenator circuit was always definitely greater and in one case reached

TABLE IV. "Crossing-over" experiment.

Exp. 365. Dog's lungs. Defibrinated blood, twice filtered. 450 c.c. in each circuit. Lungs 110 g. No œdema.

	Lung circuit							
							Mg./100 c.c.	
Hour	Temp. °C.	Pulmonary artery pressure mm. Hg	Flow c.c./min.	CO_2 content of blood c.c./100 c.c.	*p*H of blood	Hæmolysis p.c.	Glucose	Lactic acid
0.00	38·3	21	274	3·7	7·1	0·6	126	71
1.00	38·3	20	274	1·8	8·5	0·8	96	96
2.00	38·5	20	250	—	8·4	1·0	67	129
2.01			Switch over bloods					
2.18	38·5	20	275	—	8·36	18·2	85	126
3.18	38·3	25	300	—	8·30	15·6	58	141
4.18	37·8	18	126	1·40	7·82	16·1	25	166

	Oxygenator circuit						
						Mg./100 c.c.	
Hour	Temp. °C.	Flow c.c./min.	CO_2 content of blood c.c./100 c.c.	*p*H of blood	Hæmolysis p.c.	Glucose	Lactic acid
0.00	37	136	9·1	7·3	6·6	142	64
1.00	37·2	200	1·4	8·48	19·2	125	82
2.00	37	150	—	8·4	19·6	106	104
2.01			Switch over bloods				
2.18	37·5	158	—	8·28	7·0	78	129
3.18	37·5	120	—	8·37	13·3	64	134
4.18	37·5	111	1·4	7·85	15·2	57	148

about 20 p.c. in 2 hours. Since hæmolysed blood shows a slower rate of glycolysis [Irving, 1926; Reid and Narayana 1931], it might therefore be thought that the slower change in the oxygenator circuit was due to the corpuscular damage. Alternatively, it might be thought that the more rapid effect in the lung might in some way be due to a glycolytic agent passing out once and for all from the lung into the blood in the pulmonary circuit; to eliminate this the control blood was usually drawn from the reservoir after having been circulated for some time through the lungs, and was found to show no acceleration of glycolysis. We answered both of the objections further by the "crossing-over" experiment, using the arrangement shown in Fig. 1.

In this experiment, after a period in which two portions of blood from a common stock were circulated respectively through oxygenator and lung circuits, these were crossed over, so that nearly all the blood formerly traversing the lungs now went through the oxygenator circuit and *vice versa* (Table IV).

Variation in amount of lung tissue, or of blood.

In some of the experiments the perfusion was begun with both lungs, and after one or more periods one lung was tied off, the rate of flow through the remaining lung being kept the same as that previously through both. This led to a reduction in the rate of change in two experiments out of three (the remaining one showing no change), though the reduction was never proportional to the amount of lung removed. The following, in which the flow was a large one, was the most satisfactory experiment:

Exp. 384. Defibrinated blood. Initial weight of lungs, 108 g. Preparation ventilated with 5·6 p.c. CO_2 in air. Pulmonary artery pressure 53–56 mm. Hg. Temp. 38° C. Flow 500–560 c.c./min. Volume of blood in circuit 480–465 c.c. CO_2 content of serum 30 vol. p.c.: *p*H 7·67.

Hour	Glucose mg./100 c.c. blood	Lactate mg./100 c.c. blood	Exchange by lungs, mg./hour	
			Glucose	Lactate
0	87	31	—	—
1	51	52	135	63
One lung = 69 g. removed without stopping circulation through the other.				
2	26	74·5	79	47

The fact that the reduction in the rate of chemical change brought about by the removal of half the lung tissue was less than expected suggested that the action of the lung tissue might in part be of a catalytic nature and take effect for some seconds or minutes on the whole volume of blood. We have not proceeded further with this aspect of the problem, though we have noted that the total change did also vary somewhat when the total blood volume was altered.

The addition of fresh blood appeared to have a definite stimulating effect on the rate of glycolysis, but it had a similar effect when used to replace, instead of to augment, the blood initially circulating. As it seemed possible that new blood accelerated the process by reason of its higher glucose and lower lactate content, we have tried the effect of additions of glucose or of sodium lactate to the circulating blood. The results, after allowing due time for distribution of the added substances, did not indicate that any effect was produced by either addition.

Effect of varying rate of flow.

It was expected that alteration in the rate of blood flow through the lungs would alter the rate of glycolysis effected in the blood. In practice this was only partially realized, due partly to the fact that the experiment cannot readily be planned to give unequivocal results because of the natural decline in glycolysis which occurs in the course of all the experiments; hence if we begin with a large flow, glycolysis will in any case tend to fall even if the flow remains constant, whereas if we start with a small flow, any augmenting effect of increased flow will be to some extent obscured by the same causes. Curiously enough, the effect of alteration of rate of flow, between reasonable limits, and until the glucose fell to a low level, seemed to have little effect on the rate of change in the blood, as the following three experiments (cf. Table V) show. When the blood

TABLE V. Alteration in rate of blood flow.

Exp. 370. Defibrinated blood. Temp. 36–38·2° C. Hæmolysis 0·8–1·6 p.c.
Rate of glycolysis in control = 11 mg. glucose and 12 mg. lactic acid/100 c.c./hour.

Time	Pulmonary artery pressure mm. Hg	Flow c.c./min.	Glucose mg./100 c.c. blood	Lactate mg./100 c.c. blood	Mg. per hour by lung alone: Glucose	Mg. per hour by lung alone: Lactate
0	22	137	124	89	—	—
1	22	150	90	112	80	38
2	22	150	54	128	81	13
3	36	280	18	155	49	27
4	22	137	4	160	8	—
Exp. 371. Defibrinated blood. Temp. 38·4–38·8° C. Hæmolysis 0·6–10·0 p.c.						
0	33	500	127	49	—	—
1	33	500	84	92	81	98
2	12	225	44	117	68	34
3	40	600	7	147	43	37
Exp. 376. Defibrinated blood. Temp. 36·6–38° C. Hæmolysis 1·0–1·6 p.c.						
0	50	600	165	54	—	—
1	50	600	126	78	66	27
2	25	300	93	103	45	28

sugar was nearly exhausted, however, then the rate of formation was greatly reduced by retarding the flow (Exp. 370), nor could the falling off be prevented by increasing the flow (Exp. 371).

Effect of ventilation with CO_2 mixtures.

The results of seven experiments in which the lungs were ventilated for the first hour with carbon dioxide mixtures with air or oxygen, are shown in Table VI.

TABLE VI. Ventilation of lungs with CO_2 mixtures (first hour).

			Mg./100 c.c./hour			Per 100 g. lungs alone per hour		Control mg./100 c.c./hour	
Exp.	CO_2 content of gas	Blood vol.	Glucose loss	Lactic acid gain	Wt. of lung g.	Glucose	Lactic acid	Glucose loss	Lactic acid gain
374	7·6—O_2	330	13	11	101	3	7	12·0	9·0
375	5·5—O_2	390	39	24	80	52	56	13·3	12·6
382	4·0—O_2	480	24	13	108	44	4	14·0	12·1
383	7·5—air	440	24	12	90	62	23	11·3	6·3
384	5·6—air	470	36	21	108	122	58	8·0	7·7
386	7·6—air	460	30	13	110	95	28	7·3	6·4
406	4·0—air	500	27	14	90	117	47	6·0	5·5
Mean	6·0 p.c.	438	27·6	15·5	98	77·5	31·2	10·3	8·5

It will be noticed that the rate of formation of the lactate from blood sugar by the lungs, as well as that *in vitro*, is reduced as compared with experiments in which ventilation was with air. The mean of the latter (Table II) gave for the first hour 112·5 mg. glucose and 52·5 mg. of lactic acid per 100 g. lung, while the lungs ventilated with CO_2 gave an average of 77·5 and 31·2 mg.: the reduction in the rate was 31 p.c. for glucose and 40·5 p.c. for lactate. The glycolysis *in vitro* was, in air (Table II) 15·2 mg. glucose and 15·4 mg. lactic acid, and in CO_2 mixtures 10·3 mg. glucose and 8·5 mg. lactic acid: the reduction in rate brought about by

Exp. 406. Defibrinated blood. Initial weight of lungs, 90 g. Heart 89 g. Experiment began with ventilation with 4 p.c. CO_2 in air. Flow 470 c.c./min. Pulmonary artery pressure, 56 mm. Hg. Average blood volume = 500 c.c.

		Change mg./100 c.c./hour				Change per hour per 100 g. lung		
		Blood of lung circuit		Control blood				
Time hours	Ventilation	Glucose	Lactic acid	Glucose	Lactic acid	Glucose	Lactic acid	Remarks
0–1	4 p.c. CO_2	27	14	6·0	5·5	117	47	Hb = 100 p.c. Blood CO_2 = 24·5 c.c./100 c.c.
1–2	,,	22	17	6·0	5·5	89	64	O_2 sat. of blood = 93 p.c
2–2½	Air	46	32	15·0	13·0	155	95	Hb = 109 p.c.

CO_2 at an average of 6 p.c. was 33 p.c. for glucose usage and 45 p.c. for lactate formation. It would seem, from these very approximate figures, that the reduction in the rate of glycolysis brought about by CO_2 ventilation affects that in blood and lungs to about the same extent and that in both cases the lactate formation is inhibited more than is the glucose usage.

The striking increase in glycolysis when air is substituted for CO_2 mixtures is seen in the protocol (Exp. 406) which is given above (p. 53).

Possible bacterial contamination.

The perfusion apparatus was always sterilized by heat, dry or moist, according to suitability. In the earlier experiments, however, one part of the pump, the rubber bladder, was not detachable, and was merely scrubbed clean. On bacteriological examination of the blood, it was found in one experiment that there were initially 200 organisms per c.c., but after 2 hours' perfusion there were at least 10,000 mainly hæmolytic streptococci producing acid but no gas from glucose or lactose. We accordingly altered the arrangement of the pump to that shown in Fig. 1 and thereafter the entire apparatus was heat sterilized. Working with aseptic precautions throughout we then easily obtained completely sterile blood (no colonies in 1 c.c.) even after several hours of perfusion. The results were in no way altered thereby: Exps. 371, 372 and 373 are examples of experiments in which the blood was quite sterile throughout.

Volume of blood in circulation.

One of the difficulties attending quantitative estimations in lung perfusion experiments is that of the determination of the quantity of blood which is present. In all our later experiments measured amounts of blood were transferred to the graduated reservoir to begin with, and the volume of the tubes and other parts of the apparatus was known (usually 117 c.c.). To commence, therefore, the volume of blood in the reservoir (R) would be the volume (M) measured in, minus the volume of the tubes, etc., (dead space) D, and that of the lung vessels when filled with blood (L), *i.e.* $M=R+D+L$. All subsequent removals or additions of blood in the course of an experiment were measured, so that the volume present at any time was known. The volume (L) at the start of an experiment was from 20 to 50 c.c. according to size of lungs. Thus, in one experiment, 297 c.c. of blood were measured into the apparatus, and after filling all tubes and the lung vessels (at zero pressure) the reservoir contained 140 c.c. blood, the dead space was 117 c.c. and hence the lung vessels

had taken and held 40 c.c. We have always stopped the perfusion pump, but not the respiration pump, for some 20 sec. before taking the reading of the reservoir, because when the perfusion is in progress (pulmonary artery pressure 30–60 mm. Hg) the lung vessels hold 30–50 c.c. more blood than when the flow is arrested, and the vessels allowed to drain for 20 sec. into the reservoir.

During an experiment there is almost invariably a steadily increasing difference between the volume of blood known to be present in the circuit and that which would be calculated from the reservoir reading, on the assumption that the volume of blood in the lungs had remained constant throughout. The loss of volume, if due to evaporation or to passage of œdema fluid into the lungs, would be revealed by an increase in the hæmoglobin content of the blood, the two causes being distinguishable from the change in weight of the lungs. An approximate balance of fluid can be obtained only if great care is taken by ligation, as in the following instance, to prevent drainage of blood away from the lungs before weighing after these are removed from the apparatus.

Exp. 389. Perfusion for 2 hours 35 min. Volume of blood initially required to fill vessels = 30 c.c. (32 g.). Initial volume of blood in circuit = 470 c.c. Œdema at end of experiment.

Final weight of lungs	= 153 g.	Final actual blood vol.	= 426 c.c.
Initial weight of lungs	= 56 g.	Final apparent blood vol.	= 325 c.c.
Increase	= 97 g.	Volume lost	= 101 c.c. = 106 g.

Initial Hb = 100.
Final Hb = 105.

Loss of fluid calculated from Hb = 21 g.

Evaporation.		Plasma loss as œdema.	
Volume lost	= 106 g.	Total water loss	= 21 g.
Increase in lungs	= 97 g.	Evaporation	= 9 g.
Evaporation	= 9 g.	Œdema loss	= 12 g.

Gain in weight of lung.

Œdema	= 12 g.
Initial blood capacity	= 32 g.
Extra blood retained	= 53 g.
Total	= 97 g.

In the following example no œdema occurred:

Exp. 381. Perfusion for 2 hours 42 min. Initial volume of blood in circuit, 415 c.c. Volume to fill up lung vessels at zero pressure = 48 c.c.

Final weight of lungs	= 103 g.	Final actual blood vol.	= 340 c.c.
Initial weight of lungs	= 90 g.	Final apparent blood vol.	= 310 c.c.
Increase	= 13 g.	Volume lost	= 30 c.c. = 32 g.

Initial Hb conc. = 100.
Final Hb conc. = 97.

No loss by evaporation or œdema, but a calculated gain of water = 10 g. (?).

Difference 32 + 10 − 13 = 29 g. probably represents errors of measurement and loss of blood on removing lung.

Not all the experiments gave such good balance sheets as these two, but it was evident that the loss of fluid from the circuit was often greater than could be calculated from the rise in the hæmoglobin concentration of the blood. Therefore the increase of weight of the lung as shown in Table VII is often greater than would be calculated from the assumption

TABLE VII.

Exp.	Duration hours	Initial weight of lung	Actual vol. blood in circuit at Start	Actual vol. blood in circuit at End	Apparent vol. blood in circuit at Start	Apparent vol. blood in circuit at End	Apparent loss of blood c.c./100 g. lung/hour	Actual increase in wt. of lung g./100 g./hour	Diff. = prob. loss by evap. g./100 g. lung/hour	Total loss of fluid g./100 g. lung/hour from blood, calc. from Hb	Remarks
373	4·25	80	297	227	297	207	6·0	4·1	1·9	9·1	—
375	5·0	80	412	306	412	213	23·2	11·5	11·7	14·8	—
381	2·67	90	415	340	367	262	12·5	5·4	7·1	0·0	—
382	4·5	108	520	432	520	350	16·8	15·1	1·7	9·7	—
383	4·84	90	465	453	465	425	6·3	2·2	4·1	—	—
386	5·0	110	470	338	470	305	6·0	6·4	−0·4	8·7	—
374	3·0	101	390	315	390	210	35·0	23·7	11·3	—	Œdema
389	2·5	56	470	426	475	330	73·0	69·0	4·0	14·3	Œdema
397	1·5	77	450	448	420	190	223·0	202·0	21·0	74·0	Copious œdema
406	2·75	90	520	485	520	400	34·5	57·5	23·5	16·2	Copious œdema

that this is due only to passage of plasma from the blood; it seems to be due to a steadily growing tendency for whole blood to remain in the lungs, or alternatively, for there to be simultaneously a deposition mainly of corpuscles in one part (hypostatic congestion) and an outward passage of plasma as œdema fluid in other parts. Although this alternative may to some extent be held to account, as is shown by the fact that in some cases the hæmoglobin content of the circulating blood actually falls slightly, we think it more likely that there is for some reason a tendency, which increases as the experiment progresses, for the lung vessels to become distended and to drain very slowly; thus, blood will continue to drain away for many minutes after removal from the apparatus of a lung which has been perfused for 2 or 3 hours if its vessels are left freely open and particularly if it is handled, whereas after a short period of perfusion drainage is prompt.

In view of these facts it becomes, especially in later periods of a series, a matter of some importance to decide which volume of blood, the real or the apparent, should be used in calculating the chemical changes which take place on perfusion. In one experiment we attempted to determine the blood volume by the addition of a known amount of Chicago blue; the result was unsatisfactory and gave a volume somewhat

less than the apparent one. We decided, nevertheless, to use the actual volume, as measured into the circuit, as a basis for calculation, because we thought that the whole mass of lung and blood was probably in diffusion equilibrium. This appears, as a first approximation, to be the case, for if glucose or lactic acid be added to the perfusion blood, the rise of its concentration is usually only slightly more than would occur if the substance were equally distributed between lungs and blood. This is illustrated by the data of Table VIII; allowance was made for the rate of usage of glucose or formation of lactic acid by the lung itself during the time intervening between addition and analysis.

TABLE VIII. Distribution of added glucose or lactate.

					Mg./100 c.c.		
Exp.	Wt. of lung g.	Actual vol. of blood c.c.	Apparent vol. of blood c.c.	Added g.	Calc. increase if present only in apparent volume of blood	Calc. increase if present in actual volume blood + lungs	Actual increase in concentration
370	135	250	—	0·2 glucose	—	52	48
373	80	252	217	0·4 glucose	184	121	125
386	110	393	302	0·3 glucose	134	60	64
391	358 (sheep)	1130	1000	1·2 glucose	120	80	83
389	56	444	415	0·79 lactic acid, as sodium lactate	191	143	158

Graeser, Ginsberg and Friedemann [1934] also found by direct analysis that the lung only contained slightly less glucose and slightly more lactate than whole blood.

This agreement is, however, not so convincing as may appear at first sight, because we know that in dog's blood neither the glucose nor the lactate is equally distributed between corpuscles and serum. We found the corpuscles usually to have a concentration from one-third to one-eighth that of the serum as regards glucose, but about half as regards lactic acid. We cannot say conclusively what may be the distribution of glucose between serum and lung tissue, since in one case where glucose was added to a serum-perfused lung the lung appeared to retain more glucose per unit weight than did the serum; in a saline-fed lung the reverse was the case. Moreover, the following data (Table IX) show that lung œdema fluid, which is presumably also in equilibrium with lung tissue, contains a higher concentration of glucose than whole blood, but less than serum. Its lactate content is apparently about the same as

that of serum. It often contains hæmoglobin when copious and is then probably a mixture of blood and tissue fluid.

TABLE IX. Blood, serum and œdema fluid.

	Mg./100 c.c. whole blood		Mg./100 c.c. in serum (or saline)		Mg./100 c.c. in œdema fluid		
Exp.	Glucose	Lactic acid	Glucose	Lactic acid	Glucose	Lactic acid	Remarks
377	125	80	—	—	164	138	Œdema fluid Hb = 27 p.c.
397	56	136	89	205	60	194	—
399	65	96	114	—	93	121	Pig's lungs and blood
385	—	—	310	38	224	42	Saline perfusion

Bearing these various facts in mind it is evident that the quantitative results of lung perfusion experiments can only be approximate, and are particularly likely to be unreliable when there is a sudden onset of lung œdema; as might be expected from what has been stated above, this leads to a rapid loss of sugar from the blood as illustrated by the examples of Table X.

TABLE X. Effect of lung œdema.

		Rate of change mg./100 c.c./hour in blood		
Exp.		Glucose	Lactic acid	Remarks
374	Before œdema began	17	11	Chlorazol fast pink anti-coagulant
	After œdema began	58	14	
376	Before œdema began	42	22	Cyanide added to $N/2800$
	After œdema began	51	18	
377	Before œdema began	56	22	
	After œdema began	76	15	
397	Before œdema began	42	34	Cyanide added to $N/480$
	After œdema began	99	34	

It will be observed that in two of these experiments sodium cyanide had been added, and this seemed to be responsible for rapid lung œdema; it is only when the œdema is of very rapid onset that such striking results as the above are obtained. The lactic acid change is less affected than that of glucose. Nevertheless, we have disregarded any results which appeared to be complicated by lung œdema and in general have avoided prolonged experiments for the same reason.

Perfusion with serum or saline.

The same phenomenon of glucose usage and lactic acid formation was seen in two experiments in which the lungs were perfused with dog's blood serum; not all the red cells could be removed, as many were washed

out of the lung during the perfusion, but the cell content was never more than one-sixtieth that of blood. After a period of perfusion with serum the centrifuged corpuscles were added so as to reconstitute defibrinated blood and a further period of perfusion studied.

TABLE XI. Serum and saline perfusions.

Exp.	Perfusion fluid	Flow c.c./min.	Duration of perfusion hours	Mg./100 g. lung/hour: Glucose usage	Mg./100 g. lung/hour: Lactic acid formation	Remarks
368	(*a*) Serum	400	2	130	130	Cell count of serum during perfusion = 48,800/c.mm.
	(*b*) Serum + corpuscles	400	2	67	44	Oxygen capacity of blood = 20·5 c.c./100 c.c.
369	(*a*) Serum	485	2	56	42	Cell count of serum = 80,000/c.mm.
	(*b*) Serum	—	1	72	35	—
	(*c*) Serum + corpuscles	470	½	29	45	Equal vol. serum and corpuscles. Sugar all removed at end
379	Tyrode	375	1	31	31	Cell count in perfusion fluid = 32,000/c.mm.
385	Tyrode	600	1	33	39	Cell count in perfusion fluid = 2000/c.mm.

In both experiments there was less glycolysis when blood was used than with serum alone; no doubt this may in part have been due to the

TABLE XII.

Exp.	Species	Wt. of lung g.	Period hours	Flow c.c./min.	Mg./100 g. lung/hour: Glucose usage	Mg./100 g. lung/hour: Lactic acid formation	Remarks
387	Cat	32	½	50	122	100	Ventilated with 4·75 p.c. CO_2 in air. Plasma CO_2, 33 vol. p.c.
392	Cat	27	2	62	74	59	Air ventilation
390	Sheep	637	1	1090	34	43	Glucose, initially 27 mg./100 c.c. reduced to 4. Hæmolysis 1 p.c. Oxygen saturation 36 p.c. after 4 hours
391	Sheep	358	1	890	69	72	Glucose initially 23 mg./100 c.c. reduced to 1. Hæmolysis 0·96 p.c.
		358	1½	890	135	94	Same exp. after adding glucose. Oxygen saturation 70 p.c. Hæmolysis 1·2 p.c.

retardation of the process to which reference has already been made. In two other experiments Tyrode fluid, as free as possible from cells, was used for the perfusion.

It would appear from these results that the action is one of the lung tissue and does not depend on the presence of red corpuscles.

Other species.

Table XII shows that similar results are obtainable with the lungs of cats and sheep. Minced cat's lung also accelerated the rate of glycolysis when added to blood. Pig's lung similarly minced had no accelerating effect on glycolysis in pig's blood.

Haarmann [1932] has also found minced lung to produce lactate from glucose.

Discussion.

It is now possible, in view of the evidence that lactic acid is produced in the lung, not only more satisfactorily to explain the variations in lactate content in the blood of the heart-lung preparation, but also to show a further origin, in addition to blood glycolysis, for the lactate of the normal blood. Since the lactic acid formation by the lung shows a decline in rate after exsection, the amounts formed by the lung *in vivo* might be materially greater than those we have found under experimental conditions. We propose to investigate other sources from which lactic acid is contributed to the blood stream, but taking, in man, the lungs and blood alone it would seem that a quantity of lactic acid of the order of at least 1 g. per hour must be formed by glycolysis, the lungs forming about half the total amount.

Summary.

1. Exsected dog's lungs perfused with blood convert the blood sugar into lactic acid at the rate (for the first hour) of about 100 mg. glucose, with formation of about 50 mg. of lactic acid per 100 gm. lung.

2. The rate of glycolysis is slowed down as the perfusion proceeds.

3. The blood lactate of the heart-lung preparation (and no doubt *in vivo*) is formed partly by glycolysis in the blood stream and partly by the lungs. The lactic acid consumed by the beating heart of the heart-lung preparation is formed in both of these ways.

4. Sundry precautions necessary in lung perfusions are dealt with.

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