Summary. Ten adult, male, New Zealand albino rabbits were used to determine the percentages of body weight of blood, skin and fur, viscera, bone, brain and muscle. The brain weight is a constant percentage of body weight, the bone weight fairly constant and the other tissues vary within 6%.

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Transitory Diminution of Blood Pyruvate *in vitro*.

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In freshly drawn blood with or without added pyruvate, there occurs first a rapid fall in pyruvate content followed by a later rise.[1, 2] These changes are abolished by the addition of iodoacetate.[2]

In order to determine whether serum or blood cells are responsible for the fall in pyruvate content, freshly drawn human blood was defibrinated with glass beads and the serum was separated by centrifugation. The pyruvate content was determined by Lu's method[3] modified by the use of tungstic acid protein precipitation. Blood cells suspended in saline caused a rapid change in added pyruvate while the serum had no effect (Table I).

The rate and extent of this reaction varied with the blood cell suspensions of different subjects. It was not affected by anaerobiosis with carbon monoxide or nitrogen.

TABLE I.

Protocol: Blood was defibrinated and the serum separated. Red cells were washed once in 0.85% NaCl and resuspended in Ringer-PO_4 Buffer (0.02 M, pH 7.3) to 50% hematocrit. Both serum and cells after pyruvate addition kept at 37.5° in water bath with constant slow shaking.

	Pyruvate added, μg/ml	Theoretical content, μg/ml	Pyruvate found, μg/ml (Time after addition of pyruvate) 1 min.	10 min.	30 min.	120 min.	300 min.
Serum	83.4	84.1	84.2	83.9	84.0		84.0
Cells	21.6	22.3	12.7	8.2	7.8	23.3	

1 Wilkins, R. W., Weiss, S., and Taylor, F. H. L., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 296.

2 Bueding, E., and Wortis, H., *J. Biol. Chem.*, 1940, **133**, 585.

3 Lu, G. D., *Biochem. J.*, 1939, **33**, 249.

TABLE II.
Comparison of the Recovery of Pyruvate in Hemolyzed and Unhemolyzed Blood Cell Suspensions.

	% recovery (Time after addition of pyruvate) 1 min.	10 min.
Blood No. 1—Cell suspension	37.8	21.0
Cell suspension hemolyzed with saponin	5.3	67.5
Blood No. 2—Cell suspension	62.4	
Cell suspension hemolyzed by freezing and thawing	40.0	

When the blood cell suspensions were hemolyzed, either by the addition of saponin or by alternate freezing and thawing, the fall in pyruvate content after the addition of pyruvate occurred more rapidly and to a greater extent than in the case of the unhemolyzed cell suspensions (Table II).

These differences are probably due to the necessity for penetration of the pyruvate into the cells. This can be demonstrated by analysis of the fluid and cell portions of a suspension of blood cells after the addition of pyruvate. There was an immediate rise in pyruvate content of the fluid portion (obtained by centrifugation) followed by a slow fall. The cells showed very little rise during this time. Apparently, as the pyruvate entered the cells, it reacted and could not be recovered. Although the system or substance responsible for this change is stable in the intact cell at room temperature, hemolyzed cells which had remained at room temperature for one hour failed to show the fall in pyruvate content after the addition of pyruvate. Cells hemolyzed by the addition of dilute sulfuric acid (0.074 N) were also ineffective.

Bueding and Wortis[2] have attributed the rapid fall in pyruvate content of freshly drawn blood to the cocarboxylase content of that blood. In order to test this hypothesis, the (pooled) blood cell suspension of rats (maintained on a vitamin B_1 deficient diet until marked weight loss and polyneuritis occurred) was divided into two portions. To one was added cocarboxylase* (final concentration 3.3 mg %). After 10 minutes' incubation at 37.5°C, sodium pyruvate (23 μg pyruvic acid per ml) was added to both cell suspensions. A drop of 44% occurred in the sample with added cocarboxylase as against 39% for the control. In order to eliminate the influence of cocarboxylase penetration of the cells, the same experiment was repeated using hemolyzed cell suspensions without any

* Grateful acknowledgment is made to Merck and Company, Rahway, New Jersey, for the cocarboxylase used in these experiments.

significant differences in the result. As a further control, rats were placed on the same deficient diet with the addition of 20 mg thiamin chloride daily for an identical period of time. The pooled blood cell suspension showed a drop of 34% after addition of 27 μg pyruvic acid per ml, which is in agreement with the data obtained with the blood of the deficient rats. The hematocrit value of all blood suspensions was 50%. Hence, it is apparent that drop in blood pyruvate is independent of the cocarboxylase content.

In an attempt to determine the fate of the pyruvate, the oxygen consumption and the carbon dioxide production were studied by means of the Warburg technic.[4] In spite of the disappearance of 71 and 65 μg pyruvic acid in 2 separate experiments, no significant changes in oxygen or carbon dioxide pressures were observed.

In order to determine whether the pyruvate is converted to some other ketonic acid *in vitro*[1] a number of experiments were performed.

7.7 mg sodium pyruvate (equal to 6.16 mg pyruvic acid) were added to 80 ml of blood cell suspension containing 0.26 mg pyruvic acid. After 10 minutes' incubation at 37.5°C a sample was taken for ordinary analysis (Lu) and the remainder was utilized for the isolation of the hydrazone. The total pyruvic acid present at the start was 6.4 mg, and 1.03 mg or 16% was found by the method of Lu after incubation for 10 minutes. Three mg of the pure 2,4-dinitrophenylhydrazone of pyruvic acid was recovered gravimetrically corresponding to 0.99 mg pyruvic acid. The hydrazone melted at 213-15°C (uncorr.), did not depress the melting point of synthetic 2,4-dinitrophenylhydrazone of pyruvic acid, and gave the theoretical content of pyruvic acid by Lu's method. Accordingly, gravimetric analysis indicates that the loss is due to an actual disappearance of pyruvic acid, and not due to the conversion of some other keto acid which might have a smaller color value in Lu's method.

Total keto acids were assayed by the method of Lu, extracting the hydrazone with 15% sodium carbonate and developing the color with 3 N sodium hydroxide. The total ketonic acids always check with the pyruvate determinations even when 60% of the added pyruvate had disappeared.

Methyl glyoxal, glyoxal, diacetyl (as the 3-nitro-benzohydrazides), β-ketonic acids (method of Edson[5]), and acid and alkali hydrolysable derivatives of pyruvic acid could not be demonstrated.

Determinations of lactic acid[6] revealed no change during an in-

[4] Dixon, M., *Manometric Methods*, Cambridge University Press, 1939.

[5] Edson, N. L., *Biochem. J.*, 1935, **29**, 2082.

[6] Elgart, S., and Harris, J. S., *Ind. Eng. Chem., Anal. Ed.*, 1940, **12**, 758.

terval when the pyruvic acid content decreased from 5.75 to 3.58 mg %. In another experiment the lactic acid rose 0.4 mg % while the pyruvate concentration fell 2.54 mg %. This is in accord with the findings of Flock, Bollman, and Mann[7] that sodium pyruvate incubated with blood does not cause any change in lactic acid content.

It has been reported[2] that potassium cyanide increases the magnitude of the disappearance of pyruvate due to a potentiation of cocarboxylase action. However, as we have already demonstrated, the disappearance of pyruvate does not depend upon cocarboxylase content. Furthermore, the addition of the same amount of cyanide (final concentration of 0.5% sodium cyanide) to standard solution of pyruvate or to serum as well as to blood containing added pyruvate results in an almost complete disappearance of pyruvate. This may occur either from polymerization of the pyruvate or from cyanohydrin formation.

Conclusions. 1. The disappearance of pyruvate added to blood *in vitro* is caused by the blood cells and not the serum. 2. Hemolysis of the cells by saponin or freezing increases the speed and extent of this reaction. 3. The extent and rapidity of the reaction is not altered by Vitamin B_1 deficiency of the cell donor, nor by the addition of cocarboxylase to the cells *in vitro*. 4. Anaerobiosis has no effect on the reaction. 5. The reported effect of cyanide upon the reaction is due to an artefact. 6. The pyruvate which disappears is neither decarboxylated nor changed to other ketonic acids or lactic acid.

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Electro-Magnetic Measurement of Blood Flow and Sphygmomanometry in the Intact Animal.

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The electro-magnetic method of measuring blood flow[1] has been discussed in detail in previous publications.[2, 3] In this communication we wish to describe a modification which we believe to be

[7] Flock, E., Bollman, J. L., and Mann, F. C., *J. Biol. Chem.*, 1938, **125**, 49.

[1] Kolin, A., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 53.

[2] Katz, L. N., and Kolin, A., *Am. J. Physiol.*, 1938, **122**, 788.

[3] Kolin, A., PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 235.
Additional references may be found in 3.