FFA at the start of the exercise, without significant change of the plasma protein, inorganic P and glucose levels, shows that the decrease of FFA cannot be explained by a dilution of the plasma, or by a sudden release of insulin. From the 2 metabolic parameters which change rapidly during work, namely O_2 uptake and lactate level, the effect of increased metabolism was excluded by the experiments carried out on resting dogs with lactate infusions.

In view of the demonstrated interrelationship between blood lactate and plasma FFA, it would be advisable, whenever a change of blood lactate is to be expected, to measure the concentration of lactic acid in the blood in addition to the FFA. At low resting levels of blood lactate a slight rise, for instance from 7 to 20 mg/100 ml, causes a considerable drop in FFA, from 1.0 to 0.55 meq/l. Whether this is the result of a competition between the lactate anion and FFA for the albumin carrier, or a direct metabolic effect on the release of FFA in the adipose tissue, remains to be shown.

Summary. Treadmill experiments were carried out on dogs with indwelling arterial and venous catheters. The plasma free fatty acids level rapidly decreases during exercise, due to the rise of the blood lactic acid concentration, as was shown by lactic acid infusions in resting dogs. A logarithmic correlation was found between plasma free fatty acid and blood lactic acid concentration.

1. Fredrickson, D. S., Gordon, R. S., Jr., *Physiol. Rev.*, 1958, v38, 585.
2. Fritz, I. B., *ibid.*, 1961, v41, 52.
3. Andres, R., Cader, G., Zierler, K. L., *J. Clin. Invest.*, 1956, v35, 671.
4. Issekutz, B., Jr., Spitzer, J. J., PROC. SOC. EXP. BIOL. AND MED., 1960, v105, 21.
5. Friedberg, S. J., Harlan, W. R., Jr., Trout, D. L., Estes, E. H., Jr., *J. Clin. Invest.*, 1960, v39, 215.
6. Issekutz, B., Jr., Rodahl, K., *J. Appl. Physiol.*, 1961, v16, 606.
7. Dole, V. P., Meinertz, H., *J. Biol. Chem.*, 1960, v235, 2595.
8. Ström, G., *Acta Physiol. Scand.*, 1949, v17, 440.
9. Mehl, J. W., *J. Biol. Chem.*, 1945, v157, 173.
10. Chen, P. S., Jr., Toribara, T. Y., Warner, H., *J. Anal. Chem.*, 1956, v28, 1756.

Received February 12, 1962. P.S.E.B.M., 1962, v110.

Action of Plasma and of Plasma Albumin on Palmitate-1-C^{14} Oxidation by Blood Cells.* (27479)

JOSEF P. HRACHOVEC (Introduced by G. Y. Shinowara)
Department of Physiology and Biophysics, New York University School of Medicine, New York City

Plasma albumin appears to have an important regulatory function in cell metabolism. Thus, Fritz *et al.*(6) observed that plasma albumin stimulates the oxidation of palmitate-1-C^{14} by skeletal muscle *in vitro* in concentrations lower than one micromole of albumin per 7 micromoles of palmitate, but inhibits this oxidation at higher concentrations. Helinski and Cooper(8) observed that plasma albumin is an essential component for oxidative phosphorylation of sucrose, succinate, beta-hydroxybutyrate and some other substrates by rat liver mitochondria, and Sacktor *et al.*(13) made similar observations with mitochondria from insect flight muscle. Glinos (4) observed that the albumin level in blood plasma of the rat and its changes after partial hepatectomy constitute an important regulatory factor for liver growth and regeneration.

The aim of the present investigation was to obtain quantitative data concerning the action of blood plasma and of plasma albumin on rate of tissue and cell metabolism *in vitro*. We chose to use blood cells and palmitate-1-C^{14} as cell-substrate system for this study

* This research was supported in part by grant Nos. H-3173 and H-4843 from U. S. Public Health Service.

because (a) observations of Fritz *et al.*(6), of Shipp *et al.*(15), and of others indicate that to some extent the same basic interactions and processes will exist between plasma albumin, fatty acids and any tissue cells, whether these are muscle, liver, heart, or blood cells; (b) none of the usual tissue cells can be freed so completely and in such a short time from the plasma albumin present in intercellular space without any particular damage to the tissue cells as blood cells can; (c) interactions between plasma albumin and long-chain fatty acids alone(5), or between plasma albumin fatty acids and red blood cells(2,7,9), have already been intensively investigated by many authors using various methods.

Nucleated erythrocytes in bone marrow are apparently capable of performing most of the metabolic reactions characteristic of nucleated cells in other tissues. After loss of the nucleus, some of these reactions are lost and others are no longer intact. However, James *et al.*(11) have observed that blood cells are able to synthesize fatty acids, Mines *et al.* (12) have reported that blood cells incorporate fatty acids into phosphatides, and Hrachovec *et al.*(10) have reported that blood cells oxidize palmitate-1-C^{14} to $C^{14}O_2$. These observations indicate that blood cells are a suitable material for our proposed study.

Methods. All experiments reported here were performed according to the same basic pattern: Washed blood cells were incubated at room temperature (around 23° to 24°C) with palmitate-1-C^{14} in Conway microdiffusion units. Into the outer well were added: (a) 2.0 ml of an approximately 10% suspension of washed blood cells, (b) 1.0 ml of a 0.04 mM solution of palmitate-1-C^{14} in Tyrode-phosphate medium, and (c) 1.0 ml of diluted blood plasma, or 1.0 ml of Tyrode-phosphate medium. Therefore the final concentrations in the total suspension medium were 0.01 mM for palmitate-1-C^{14} and approximately 5% for blood cells.

A small removable stainless steel cup containing one ml of 10% KOH solution was inserted into the center well of each of the microdiffusion units to capture the $C^{14}O_2$ evolved during incubation; all the microdiffusion units were then closed at the same time. In each experiment there were (a) 2 or 3 control units containing only palmitate-1-C^{14} and no blood cells, (b) 2 or 3 other units containing palmitate-1-C^{14} with washed blood cells only, and (c) 6 to 8 experimental units containing palmitate-1-C^{14} with washed blood cells in presence of various concentrations of blood plasma or plasma albumin.

After an incubation period of exactly 2 or 3 hours, the microdiffusion units were opened, and in each unit the removable cup (containing the evolved $C^{14}O_2$) was replaced by another cup with 10% KOH; the microdiffusion units were then closed again for a new period of incubation. Usually collections from several consecutive incubation periods were analyzed for evolved $C^{14}O_2$. The pH of the incubating Tyrode-phosphate medium was checked with the Beckman pH meter before incubation and then again after incubation and was found within the range of pH 7.2 to 7.4. On the basis of the mean data obtained, the instantaneous rate of $C^{14}O_2$ evolution was plotted against time. For each new experiment the microdiffusion units were carefully washed in cleaning solution, rinsed abundantly with double-distilled water, and dried. All the microdiffusion units were gently agitated during incubation in order to prevent sedimentation of blood cells to the bottom of the incubation wells, which would decrease the amount of $C^{14}O_2$ evolved.

The $C^{14}O_2$ evolved during each period of incubation and captured in 10% KOH was precipitated with an excess of 1 *M* $BaCl_2$ and the $BaC^{14}O_3$ was recovered and assayed for radioactivity. Isotope measurements were carried out with the Automatic Scaler Unit, model 161A, from Nuclear-Chicago Corp.; the background was about 16 to 18 counts per minute. Radioactivity was usually measured as the time required for 6,400 counts.

Preparation of blood cells. The blood of normal, adult, fasting male rats weighing between 300 and 400 g and killed by decapitation, was collected into about 10 ml of modified Tyrode-phosphate medium, prepared with double-distilled water (NaCl – 0.137 M; KCl – 0.004 M; Na_2HPO_4 buffered to pH 7.4 – 0.01 M), and containing 10 units of heparin. The collected blood was centrifuged

TABLE I. Effect of Homologous Blood Plasma on Oxidation at Room Temperature of 0.01 mM Palmitate-1-C^{14} by Blood Cells (5% Concentration) Washed by the Short Procedure.

Time (hr)	Evolved $C^{14}O_2$*: Final dilutions of the added homologous blood plasma: 20×	40×	80×	160×	320×	640×	1280×	2560×	No plasma added
0-2	46 ±3	78 ± 5	124 ± 9	238 ±17	398 ±28	597 ±39	835 ±61	947 ± 77	1010 ± 85
2-4	65 ±5	115 ± 9	281 ±18	374 ±25	616 ±45	860 ±69	1096 ±89	1128 ± 92	1294 ± 97
4-6	106 ±8	194 ±15	356 ±23	540 ±39	784 ±63	936 ±75	1280 ±98	1416 ±129	1542 ±126

* Counts/min. for $BaC^{14}O_3$ precipitate, representing difference between experimental and blood cell-free control.

and the supernatant plasma was retained for later experimentation. The blood cells were then suspended in 20 volumes of Tyrode-phosphate medium and centrifuged again. After this second centrifugation the blood cells were resuspended in 20 volumes of the same medium and divided into 2 equal portions.

With the first portion a third centrifugation was performed immediately; the supernatant was discarded and the cells were suspended and centrifuged again in 20 volumes of the same medium. After this fourth centrifugation, a 10% suspension of washed blood cells was prepared, which then was incubated with palmitate-1-C^{14} and also used for an estimate of the erythrocyte concentration by a photometric determination of total hemoglobin. In all experiments related in Table I the blood cells were washed in this way.

The second portion of blood cells was treated in exactly the same way as the first one, except that the cells were left in the Tyrode-phosphate medium for 24 hours before the third centrifugation. In all experiments related in Table II the blood cells were washed in this way.

Preparation of the substrate. Palmitic-1-C^{14} acid (specific activity 1.99 millicuries/millimole, from Nuclear-Chicago Corp.) was stored dissolved in benzene. An aliquot of the benzene solution was then pipetted into a small volumetric flask and evaporated in a mild stream of air. Palmitic acid is then neutralized with 0.01 M KOH solution in slight excess, dissolved at 75°C and adjusted with Tyrode-phosphate medium to the concentration of 0.04 mM and pH 7.4. Aliquots of this solution are used for incubation with blood cells; a new solution of palmitate-1-C^{14} is prepared for each experiment.

Results. It was repeatedly verified that the amount of the evolved $C^{14}O_2$ is the same in all the microdiffusion units, if exactly the same amount of blood cells is incubated with exactly the same amount of palmitate-1-C^{14}. If, however, inhibitory substances such as diluted blood plasma are added to some of the microdiffusion units, then the amount of $C^{14}O_2$ evolved decreases in those units, as compared to the amount evolved in units where no inhibitory substances are added. Each of the values given in the accompanying tables is the mean of 8 to 10 determinations.

Table I presents data on oxidation of 0.01 mM of palmitate-1-C^{14} by 5% suspensions of blood cells washed by the short first procedure, and incubated for 3 two-hour periods in the presence of 8 different dilutions of homologous blood plasma. Comparison of the first and ninth columns in Table I shows that washed blood cells oxidize palmitate about 20 times faster in the absence than in the presence of 20-times diluted blood plasma. With higher dilutions of blood plasma, higher rates of palmitate oxidation are observed, but even with 1280-times diluted blood plasma (and possibly with higher dilutions) the rate of palmitate oxidation is still not as high as when no plasma at all is added.

Table II presents data from experiments similar to those in Table I, except that the time lag between the second and third centrifugations was 24 hours for the experiments of Table II, whereas it was about 15 minutes for the experiments of Table I. Comparison of the data in the last columns of Tables I and II shows that the mere suspension of washed blood cells in isotonic medium for 24 hours has nearly doubled their initial rate of palmitate oxidation. This might be explained by

TABLE II. Effects of Homologous Blood Plasma on Oxidation at Room Temperature of 0.01 mM Palmitate-1-C^{14} by Blood Cells (5% Concentration), Washed by the 24 Hours Procedure.

Time (hr)	Evolved $C^{14}O_2$*: Dilutions of the added blood plasma in the final suspension: 20×	40×	80×	160×	320×	640×	1280×	2560×	No plasma added
0-2	29 ± 2	48 ± 4	90 ± 7	187 ± 16	414 ± 31	748 ± 59	1230 ± 95	1510 ± 119	1964 ± 158
2-4	41 ± 3	63 ± 5	178 ± 32	764 ± 137	1740 ± 215	2420 ± 267	2840 ± 241	2640 ± 209	2317 ± 183
4-6	67 ± 8	147 ± 19	987 ± 169	2579 ± 233	3215 ± 258	2390 ± 211	1875 ± 155	1716 ± 133	1610 ± 126

* Counts/min. for $BaC^{14}O_3$ precipitate, representing difference between experimental and blood cell-free control.

inhibitory factors that are bound to the blood cells and leave them very slowly during washing, *i.e.*, over a period of several hours.

Table II further shows that in the period of incubation between 2 and 4 hours the rate of palmitate oxidation is highest when 1280-times diluted plasma is added, and decreases to the right (with higher dilutions of blood plasma), as well as to the left (with lower dilutions of blood plasma). These maximal values become even more pronounced during the period between 4 and 6 hours when the rate of palmitate oxidation is highest with the 320-times diluted plasma and again decreases at both higher and lower concentrations. A possible explanation of these observations might be that (a) the substances in blood plasma that are inhibitory in high concentrations are stimulatory in low concentrations; (b) these substances are in equilibrium with the same substances bound *in vivo* to blood cells in such a high amount (and diffusing *in vitro* into the outside medium so slowly) that this amount does not decrease below inhibitory level even after repeated washing, unless the washed blood cells stay for several hours in isotonic medium; (c) even after a new addition of these substances in stimulatory concentration (as in experiments of Table II, when 1280-times, 640-times and 320-times diluted plasma was added) it takes several hours for their stimulatory action to come to full effect.

Table III presents data from experiments on oxidation of 0.01 mM palmitate-1-C^{14} by a 3% suspension of blood cells washed by the short procedure and incubated for 4 three-hour periods in the presence of 4 different concentrations of bovine plasma albumin. The data show that addition of plasma albumin in increasing concentrations decreases the rate of palmitate oxidation by washed blood cells. Moreover, in all 5 columns of Table III the amount of evolved $C^{14}O_2$ reaches its maximal values either in the period of incubation between 3 and 6 hours or between 6 and 9 hours, then decreases again during the next period of incubation.

Experimental data from Table III were used for construction of curves 1 to 5 in Fig. 1, representing the instantaneous rate of

TABLE III. Effect of Bovine Plasma Albumin on Oxidation at Room Temperature of 0.01 mM Palmitate-1-C^{14} by Blood Cells (3% Concentration) Washed by the Short Procedure.

	Evolved $C^{14}O_2$*				
	Concentrations of albumin in mM:				
Time (hr)	.08	.02	.005	.00125	No albumin added
0- 3	135 ± 11	356 ± 29	634 ± 43	890 ± 59	1085 ± 73
3- 6	715 ± 53	1940 ±138	2275 ±147	2570 ±153	2787 ±129
6- 9	1994 ± 95	1785 ± 83	1560 ± 91	1419 ± 75	1234 ± 79
9-12	1385 ± 68	812 ± 71	505 ± 49	327 ± 35	209 ± 26
	Ratio of albumin in mM to palmitate in mM				
	8 : 1	2 : 1	½ : 1	⅛ : 1	0 : 1

* Counts/min. for $BaC^{14}O_3$ precipitate, representing difference between experimental and blood cell-free control.

$C^{14}O_2$ evolution, and data from Table I for construction of curves a to d, also in Fig. 1. The same construction procedure illustrated with curve 2, based on data from the second column of Table III, was also applied for all the other curves; dotted lines are extrapolations.

Dervichian(3) observed that 100 g of blood cells can rapidly adsorb 200 to 400 mg of fatty acids from the solution before hemolysis takes place. In our experiments, the ratio of 0.01 mM palmitate to 5% suspension of blood cells is only about 1½ to 3% of this saturation ratio. The fact that the rate of $C^{14}O_2$ evolution rises rapidly to its maximal value, as Fig. 1 demonstrates, would then appear to be due to rapid adsorption of the palmitate molecules on washed blood cells, constituting the first step of their oxidation, which then starts for all the molecules at approximately the same time. The subsequent early decline in $C^{14}O_2$ evolution is explained by the fact that only the first carbon of the palmitate molecules is labeled, whereas the whole molecule can be used as a source of energy and gradually cleared of acetyl fragments until completely degraded.

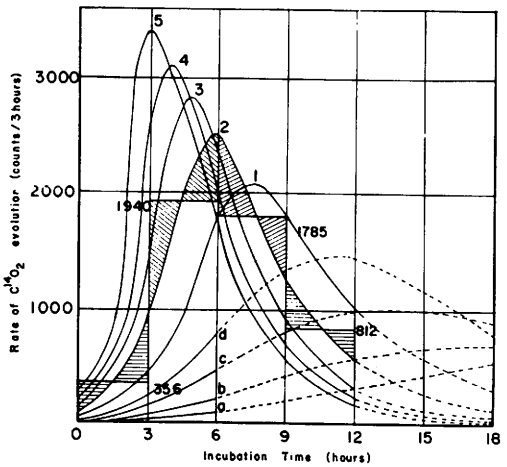

FIG. 1. Curves of instantaneous rate of $C^{14}O_2$ evolution during incubation of palmitate-1-C^{14} with washed blood cells, as constructed from data in the 5 columns of Table III, for curves 1 to 5 and in the first 4 columns of Table I, for curves a to d. The construction process is illustrated with curve 2, based on data from the second column of Table III; dotted lines are extrapolations. All curves are corrected to a blood cell concentration of 5%.

When the washed blood cells are incubated with palmitate-1-C^{14} in the absence of plasma albumin (or of blood plasma or any other inhibitory substances), then the peak of $C^{14}O_2$ evolution is high and is reached in a very short time (curve 5, Fig. 1), indicating that in the absence of inhibitory substances the speed of palmitate oxidation is very rapid. In the presence of plasma albumin in increasing concentrations, the speed of palmitate-1-C^{14} oxidation gradually decreases; consequently, the peak of $C^{14}O_2$ evolution also decreases and is reached after an increasingly longer time (curves 1 to 4, Fig. 1).

Discussion. Our results show that (a) blood plasma, even when diluted several hundred times, has an inhibitory action on palmitate oxidation by washed blood cells; (b) plasma albumin is one of the substances contributing to this inhibitory action of blood plasma; (c) under certain experimental conditions and with very high dilutions of blood plasma, its inhibitory action is replaced by a stimulatory one.

The first step of the action of albumin on

palmitate oxidation by blood cells appears to be closely related to the "protein-coating" of blood cells. Thus, Hewitt(9) observed that the so-called "anti-sphering factor," the presence of which on the red cell surface is necessary for its disk shape, is identical with the carbohydrate-poor fraction of plasma albumin, and that its removal by adsorption changes red cell disks into spheres, whereas the carbohydrate-rich fraction of plasma albumin shows very little activity. Furchgott (7) observed that this active, carbohydrate-poor fraction of plasma albumin can be deprived of its "anti-sphering activity" by addition of traces of palmitic acid or oleic acid. Furchgott(7) also observed that plasma albumin is in equilibrium between blood plasma and the red cells, which take up plasma albumin to the extent of approximately 800 mg per 100 ml of cells; when the plasma is washed away, this albumin goes into a new equilibrium between the cells and the saline medium. This is in accordance with earlier observations by Scott(14) that dilution of blood with an isotonic medium causes the blood corpuscles to lose protein as well as non-protein nitrogen. Later Bellis and Scott (1) found that upon addition of isotonic saline to whole blood *in vitro,* total protein of the plasma became greater than it was in the original plasma, indicating that some protein entered the blood plasma from the corpuscles.

Fatty acids are known to have hemolytic action, but the concentrations of palmitate-1-C^{14} (0.01 mM) which we used in our metabolic studies are only about 2% of the minimum concentrations of fatty acids required to produce hemolysis at pH 7.4(3). Davis and Dubos(2) observed that plasma albumin protects washed red blood cells against the hemolytic action of oleic acid, and that each molecule of albumin binds about 3 to 6 molecules of oleic acid tightly enough to prevent this hemolytic action. This ratio is in accordance with later and more detailed observations of Goodman(5) who reported that there are 3 types of binding sites, with 3 different association constants, on each molecule of plasma albumin, binding 2 molecules of fatty acids very tightly, seven molecules less tightly, and 20 molecules very weakly. It is interesting that in the experiments reported here, the 320-times diluted blood plasma contains plasma albumin in about 0.0014 mM concentration, which with 0.01 mM palmitate-1-C^{14} gives the concentration ratio of approximately 1:7, and that it is at these concentrations that the maximal stimulatory effect is observed (Table II).

The regulatory action of plasma albumin in cell metabolism (inhibitory at high concentrations, stimulatory at low ones) probably is related largely to changes in its concentration equilibrium between various compartments inside and outside the cell. Thus, intracellular plasma albumin, which is an essential component for oxidative phosphorylation by cell mitochondria(8,13), appears to be in equilibrium with plasma albumin in the intercellular space. On the other hand, isotope tracer studies also indicate that albumin of blood plasma is in equilibrium with albumin in the intercellular space, where it is in part bound to the tissue cells. It can be considered that the regulatory action of plasma albumin in the cell metabolism of other substrates(8) is dependent on its concentration equilibrium between the inside and the outside of the cell, or perhaps an adsorption equilibrium between the cells and the environment, in a manner similar to its action on palmitate oxidation by blood cells.

Summary. Washed blood cells from normal, fasting adult rats were incubated with palmitate-1-C^{14} in the presence of increasing dilutions of homologous blood plasma, or in the presence of various concentrations of bovine plasma albumin. It has been observed that (a) blood plasma, even when diluted several hundred times, has an inhibitory action on palmitate oxidation by washed blood cells; (b) plasma albumin is one of the substances contributing to this inhibitory action of blood plasma; (c) under certain experimental conditions involving very high dilutions of blood plasma, its action is stimulatory rather than inhibitory. The data from several consecutive periods of incubation were used for construction of skewed bell-shaped curves of instantaneous $C^{14}O_2$ evolution; they are steep and high in the absence of plasma albumin, but flatten out in its presence. The results

are discussed in relation to the action of plasma albumin on cell metabolism and to interactions between fatty acids, plasma albumin, and red blood cells.

1. Bellis, C. J., Scott, F. H., PROC. SOC. EXP. BIOL. AND MED., 1931, v29, 1105.

2. Davis, B. D., Dubos, R. J., *J. Exp. Med.,* 1947, v86, 215.

3. Dervichian, D. G., *Ann. Inst. Pasteur* (Paris), 1950, v79, 338.

4. Glinos, A. D., in: *A Symposium on the Chemical Basis of Development,* Edited by William D. McElroy and Bentley Glass (Johns Hopkins Press, 1958), p813.

5. Goodman, D. S., *J. Am. Chem. Soc.,* 1958, v80, 3892.

6. Fritz, I. B., Davis, D. G., Holtrop, R. H., Dundee, H., *Am. J. Physiol.,* 1958, v194, 379.

7. Furchgott, R. F., *J. Exp. Biol.,* 1940, v17, 30 and 126.

8. Helinski, D. R., Cooper, C., *J. Biol. Chem.,* 1960, v235, 3573.

9. Hewitt, L. F., *Biochem. J.,* 1938, v32, 27 and 1540.

10. Hrachovec, J. P., Leblanc, M., Rockstein, M., PROC. SOC. EXP. BIOL. AND MED., 1961, v107, 205.

11. James, A. T., Lovelock, J. E., Webb, J. P. W., in: *Essential Fatty Acids,* edited by H. M. Sinclair, Butterworth Scientific Publications, London, 1957, p72; *Biochem. J.,* 1959, v73, 106.

12. Mines, C. J., Fillerup, D. L., Mead, J. F., *Nature,* 1961, v190, 92.

13. Sacktor, B., O'Neill, J. J., Cochran, D. G., *J. Biol. Chem.,* 1958, v233, 1958.

14. Scott, F. H., *J. Physiol.,* 1915, v50, 128.

15. Shipp, J. C., Opie, L. H., Challoner, D., *Nature,* 1961, v189, 1018.

Received February 15, 1962. P.S.E.B.M., 1962, v110.

Arteriovenous Pressure Differences Following Arterial Occlusion in the Intestine.* (27480)

LERNER B. HINSHAW

Environmental Physiology Branch, Civil Aeromedical Research Institute, Federal Aviation Agency, Oklahoma City, Okla.

Vascular resistance has been observed to increase markedly at low perfusion flow rates in the whole animal(1,2) and in isolated tissues(3-5). It has been postulated that as flow is further diminished, the increase in resistance may proceed to total occlusion of small arterial vessels once a critical pressure has been reached(6). Recent investigations have been concerned with the magnitude of this pressure at which blood flow is reduced to zero(7-10). Results have shown that if a positive arteriovenous pressure difference is found at zero flow it is produced by extravascular pressure acting as a collapsing force on blood vessels(8,9). It was considered of interest to extend these earlier observations to incorporate the visceral circulation.

Methods. The perfusion technics used have been previously reported(7-9,11,12). Adult dogs intravenously anesthetized with sodium pentobarbital, 30 mg/kg, were used in 8 perfusion experiments. A loop of the small intestine was carefully dissected free, together with its connection to the superior mesenteric artery and aorta, and perfused in a retrograde fashion *via* the aorta, the tip of the perfusion cannula positioned just distal to the origin of the superior mesenteric artery. This procedure permitted transfer of the intestine to the perfusion apparatus without interruption of blood flow. Great care was taken to avoid severing arterial vessels so that all blood perfused tissue and drained through a large severed mesenteric vein. Intestines were transferred to the perfusion apparatus, suspended on a strain gauge weighing device(13) and perfused with a sigma-motor pump at the highest flow compatible with the isogravimetric state. Blood for the intestinal perfusion circuit was obtained from a pump-lung apparatus(8,9), or anesthetized

* Research completed in part at Univ. of Minnesota, Dept. of Physiology, supported by grant from Life Insurance Medical Research Fund.