tachyphylaxis occurred in contrast to many of the sympathomimetic series of drugs(2). Perfusion of the coronary vessels by the drug was associated with a variable response as determined by the Rein flow meter(3).

The present investigation has shown a remarkable increase in systemic, pulmonary, and coronary vascular resistance after administration of 2 amino-4-methyl pyridine. The bradycardia is presumed to be vagal secondary to increased peripheral vascular resistance and elevated systemic arterial pressure, since it can be blocked by atropine.

Contrary to the effects of some sympathomimetic agents no increase in body or cardiac oxygen consumption was produced by administration of 2 amino-4-methyl pyridine. The reduction in cardiac output was sufficient to decrease left ventricular minute work even though calculated stroke work and systemic arterial pressure were increased. In line with reduced cardiac work and slower cardiac rate, coronary blood flow and myocardial oxygen consumption decreased. That there was no significant change in cardiac efficiency, seems particularly significant in view of the decrease in cardiac output, since work against increased pressure is reported to be more costly to the myocardium than work induced by increased flow(6). This maintenance of efficiency is most probably attributable to the decrease in cardiac rate since it has been shown that cardiac rate is inversely related to efficiency(7).

Conclusion. 1. The systemic and coronary hemodynamic effects of Ascensil®, (2-amino-4-methyl pyridine) also known as RA 1226 and in the German literature as W 45, has been investigated in 10 mongrel dogs. 2. Its administration has been associated with a marked increase in peripheral, pulmonary and coronary vascular resistance accompanied by an increase in peripheral arterial and right atrial pressure. 3. Considerable reduction occurred in cardiac output, coronary blood flow and myocardial oxygen usage per 100 g per minute, subsequent to its administration. 4. Calculated cardiac efficiency, body oxygen consumption, total body respiratory quotient and cardiac respiratory quotients were unchanged.

1. von Haxthausen, E., *Arzneimittelforschung,* 1955, v5, 370.

2. ———, *Arch. Exp. Path. u. Pharmakol.,* 1955, v226, 163.

3. Gottestein, U., Hille, H., Oberdorf, A., Schaeffer, H., *ibid.,* 1956, v228, 314.

4. Foltz, E. L., Page, R. G., Sheldon, W. F., Wong, S. K., Tuddenham, W. J., Weiss, A. J., *Am. J. Physiol.,* 1950, v162, 521.

5. Altman, P. L., Dittmer, D. S., Grebe, R. M., *Handbook of Circulation,* W. B. Saunders Co., Philadelphia & London, 1959, p355.

6. Sarnoff, S. J., Braunwald, E., Welch, G. H., Stainsby, W. N., Case, R. B., Marcruz, R., *Work and the Heart,* Paul B. Hoeber, Inc., New York, 1959, pp18-33.

7. Maxwell, G. M., Castillo, C. A., White, D. H., Jr., Crumpton, C. W., Rowe, G. G., *J. Clin. Invest.,* 1958, v37, 1413.

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Glyceride Content of Human and Canine Red Blood Cells. (26022)

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During studies on clearance of intravenously administered fat emulsions, chemically direct measurements were made of the tri-, di-, and monoglyceride content of washed red blood cells. Because of some unusual and interesting observations, it was believed desirable to report the results of these studies.

Methods. Plasma was separated from the cells of heparinized whole blood samples in a 5°C refrigerated centrifuge. The cells were washed with an equal volume of 0.9% NaCl solution, recentrifuged at 2260 g for 30 min-

TABLE I. Glyceride and Protein Bound Fatty Acid (NEFA) Content in Micromoles per Liter of Washed Red Blood Cells of Dogs (n = 8) and Human Males (n = 9).

	Dog		Human	
	Mean	S.D.	Mean	S.D.
Triglyceride	199	55	245	90
Diglyceride	413	120	247	50
Monoglyceride	124	55	149	48
NEFA	184	52	357	89
Total—μM/l	920	152	998	145
μeq/l	1,731	301	1,735	305

utes, and the liquid decanted. The buffy coat was discarded and the red cells were washed 3 more times as above. The washed red cells were then hemolyzed in 2 volumes of distilled water with mechanical agitation. When complete hemolysis had occurred, 1 ml portions were immediately placed into appropriate extraction solutions (chloroform : zeolite for glycerides; heptane : isopropanol : sulfuric acid for non-esterified fatty acids or NEFA). Tri-, di-, and monoglycerides were separated on small silicic acid columns; after alkaline hydrolysis and periodate oxidation, the glycerides were measured by the color developed with chromotropic acid(1). NEFA was determined by the method of Dole(2); lactic acid and acidic phospholipids are possibly included in this fraction(3).

Results and discussion. Tri-, di-, monoglyceride, and NEFA levels of human and canine red blood cells are given in Table I. Whereas in cells of both species the content of tri- and monoglycerides is similar, the dog cells have considerably more diglyceride and less NEFA than human red cells. In plasma of dogs fasted 20 hours total glyceride level matches that of the cells (Fig. 1); however, 75% is triglyceride and proportionately much less di- and monoglyceride occurs in the plasma than in the red cells. Human males fasted for 12 hours had twice as much total glyceride in blood serum as in red cells. Since the cell and serum contents of mono- and diglycerides are similar, it appears that almost 4 times as much triglyceride occurs in a given volume of fasting blood serum as in the red cells of humans.

Amounts of glycerides and NEFA in red blood cells fluctuate within the limits indicated by the standard deviations shown in Table I. These are relatively fixed levels and are not influenced by great elevation in fat content of the plasma. Thus, in humans 4 hours after meals containing 50 and 100 g of corn oil or cocoanut oil and also in dogs 8 to 30 minutes after being given intravenous fat emulsion, the washed red blood cell glycerides and NEFA were not significantly different from those in the fasting state.

Previously reported determinations of neutral fat in erythrocytes employed indirect procedures in which phospholipids and cholesterol were deducted from total lipid to obtain the neutral fat. By this means Erickson and associates found 51 mg per 100 g of human red cells and from 40 to 69 mg of neutral fat in 100 g of ox, sheep, or chicken red cells(4). If 277 is assumed to be the average molecular weight of the fatty acids of red cell glycerides as can be calculated for human plasma(5),

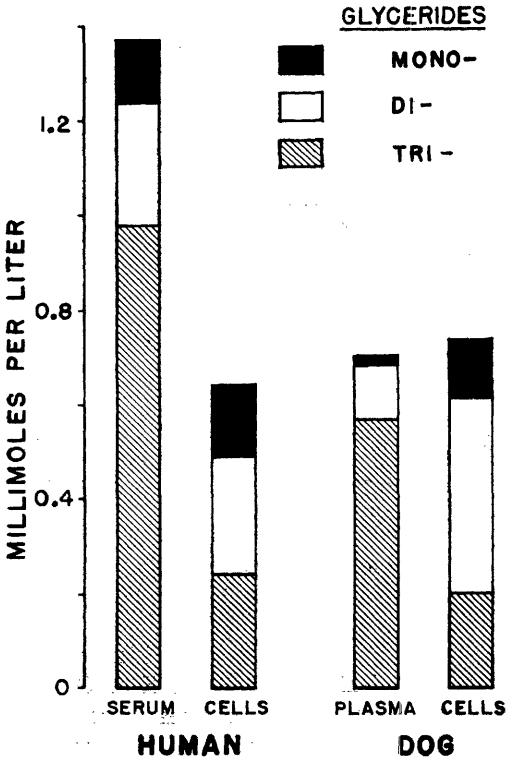

FIG. 1. Glyceride contents of red blood cells compared with that of serum and plasma in fasting humans and dogs. Human serum data represents mean values of 21 male subjects, ages 20-29 yr. Dog plasma values are mean values of 6 determinations in 3 dogs.

the present data would indicate 42 mg of total glycerides per 100 ml of cells.

Hirsch recently showed that complex mixtures of lipids can be separated quantitatively by sorption and elution from silic acid columns(6). We have modified his method so that small quantities of tri-, di-, and monoglycerides can be extracted from 1 ml blood samples, and a direct chemical determination of glyceride glycerol is then performed(7).

Ponder's experience with the cytochemistry of erythrocytes of diverse size and shape led him to express the composition in terms of number of various molecules per cell(8). Taking the volume of a dog erythrocyte as 67 μ^3 and that of the human as 90 μ^3, canine erythrocytes have 0.37×10^8 and human erythrocytes have 0.54×10^8 molecules of glycerides plus NEFA per cell. This is about one-sixth the number of hemoglobin molecules. On the basis of surface areas of 127 μ^2 and 168 μ^2, the glyceride and NEFA fatty acid residues would occur about once in each 175 square Angstroms of cell surface. A surface distribution of this intensity can be compared with the 520 $Å^2$ assigned to one molecule of tetradecylsulfate for production of spheres; "lysis occurs when there is only one molecule per 44 $Å^2$ of cell surface as a minimum"(9).

1. Vacca, J. B., Waring, P. P., USAMRNL Report No. 251, 24 Sept., 1960.

2. Dole, V. P., *J. Clin. Invest.*, 1956, v35, 150.

3. Trout, D. L., Estes, E. H., Friedberg, S. J., *J. Lipid Research*, 1960, v1, 199.

4. Erickson, B., Williams, H., Bernstein, S., Aurin, I., Jones, R., Macy, I., *J. Biol. Chem.*, 1937, v122, 515.

5. Dole, V. P., James, A. T., Webb, J. P. W., Rizack, M. A., Sturman, M. F., *J. Clin. Invest.*, 1959, v38, 1544.

6. Hirsch, J., Ahrens, E. H., Jr., *J. Biol. Chem.*, 1958, v233, 311.

7. Van Handel, E., Zilversmit, D. B., *J. Lab. and Clin. Med.*, 1957, v50, 152.

8. Ponder, E., *Hemolysis and Related Phenomena*, Greene and Stratton, 1948, p115.

9. ———, *Ann. N. Y. Acad. Sci.*, 1947, v48, 579.

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Failure to Demonstrate Anti-Hormonal Antibodies in Rats after Maximal Response to Daily Administration of Growth Hormone.* (26023)

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Nearly pure growth hormone (GH), but not pure GH, produced antibodies in rabbit and guinea pig without adjuvant(1). Other reports cited in(2) are open to question because of lack of rigor of proof of purity of GH employed. Pure GH with Freund's adjuvant produces GH-inactivating antibodies(2). Freund's adjuvant can apparently cause autoantibody formation(3), and therefore may have hapten action. Adult Norway rats treated with highly purified GH or GH-containing extracts eventually cease growing at a given dosage per unit weight irrespective of how great this dosage(4). This phenomenon (plateauing) does not result from diabetes (5), ageing of the rat(6), altered steroid production(7), or from altered cardiac output or metabolic rate(8). In the present investigation, serum from plateaued GH-treated giant rats was assayed for anti-GH activity to determine if GH antibodies, or antihormones, are involved in plateauing.

Materials and methods. Eight female Long-Evans rats, plateaued after 248 daily s.c. injections of a pH 10.5 extract of bovine anterior pituitaries(5), at a dose representing 400 mg anterior lobe tissue/kg body weight/day (equivalent to 12.8 mg GH/kg/day), served as source of "treated serum." These rats had attained a mean body weight of 633 g (2.4 times that of their controls). During the first 40 days of treatment, they gained an

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