toacetate solution had been introduced. In one rat we used a hypodermic syringe to inject an additional 2 ml into the tail vein over a period of half an hour. In all instances a total of 4 hours elapsed before the animal was sacrificed. Following this, the heart was removed, washed with saline and dropped into hot 30% KOH together with 35 mg of purified glycogen made from rat liver. The carrier material was necessary in view of the fact that a single rat heart contains only about 2 mg of glycogen which is an inadequate amount for isolation, purification and degradation. The dilution does not interfere with evaluation of radioactivity since counting was done on the total sample which was highly purified (4), degraded to CO_2 by persulfate oxidation (5) and assayed as $BaC^{14}O_3$.

Results. According to Lackey *et al.*(2) the infusion of acetoacetate can cause as much as a 60% increase in heart carbohydrate. In the average rat this represents formation of approximately 1.5 mg of glycogen over and above that normally present. We would not expect our experiments to effect this much synthesis since we introduced only about 60% as much ketone as the original workers. However, assuming that we obtained only 10% as much synthesis and assuming that no more than one C^{14} from the acetoacetate was incorporated into each glucose of the glycogen molecule, we would have expected radioactivity in the order of 500 counts per minute. In none of our experiments did the glycogen molecule contain any traces of radioactivity. We are forced to conclude therefore, that the increase in heart glycogen accompanying ketonemia does not represent a direct conversion of ketone bodies to carbohydrate. The mechanism of this effect is still an open question.

Summary. The intravenous injection of large amounts of acetoacetate-3-C^{14} has been effected under conditions reported to cause a net increase in heart glycogen. Isolation and degradation of the cardiac glycogen failed to divulge any trace of radioactivity. It thus appears that the net increase in heart carbohydrate accompanying ketonemia represents some secondary and yet undetermined influence of ketone bodies.

1. Fisher, N. F., and Lackey, R. W., *Am. J. Physiol.*, 1925, v72, 43.

2. Lackey, R. W., Bunde, C. A., and Harris, L. C., PROC. SOC. EXP. BIOL. AND MED., 1947, v66, 433.

3. Calvin, M., Heidelberger, C., Reed, J. C., Talbert, B. M., and Yankwich, P. E., *Isotopic Carbon,* p212, John Wiley and Sons, New York, 1949.

4. Lifson, N., Lorber, V., Sakami, W., and Wood, H. G., *J. Biol. Chem.*, 1948, v176, 1263.

5. Calvin, M., Heidelberger, C., Reed, J. C., Talbert, B. M., and Yankwich, P. E., *Isotopic Carbon,* p94, John Wiley and Sons, New York, 1949.

Received March 8, 1956. P.S.E.B.M., 1956, v91.

Feasibility and Safety of Frequent Plasmapheresis of the Same Human Donors.* (22347)

JOSEPH SMOLENS, JOSEPH STOKES, JR., ELIZABETH MCGEE, AND VIRGINIA HUNTER.
(Introduced by T. N. Harris.)

Children's Hospital of Philadelphia, Dept. of Pediatrics, School of Medicine, Univ. of Pennsylvania.

Plasmapheresis is the obtaining of whole blood from a donor with the retention of the plasma; the red blood cells being returned to the donor. This has been accomplished in the past in both man and other animals by well known technics(1-5). These are laborious, time consuming, and have many other obvious drawbacks inherent in the methods which would prevent large scale use. The present study is concerned with plasmapheresis in man utilizing the ADL-Cohn Blood

* This investigation was supported in part by a research grant H-1750 from the National Heart Institute of the National Institutes of Health, Public Health Service.

Fractionator.† The theoretical aspects of this technic have been discussed(6). With this equipment the red blood cells and plasma are continuously separated during the phlebotomy. It provides a sterile closed mechanical system for taking blood from a donor and separating it into many of its constituents, any of which may be returned immediately to the donor through the same needle used for the phlebotomy. The temperature of the removed blood during separation is decreased to 8°C or lower in a very short period of time which prevents adverse enzyme activity. Completely non-wettable surfaces have been utilized throughout, consisting of either polyvinyl plastic‡ or silicone-coated§ metal. A complete description of the Blood Fractionator is being prepared(7). One of the problems being investigated in the present study is to determine whether biweekly plasmapheresis of the same donor over a period of one year is safe and feasible. This project has been in progress since April 1955 (about 40 weeks) and because of the practical applications, as well as the broad theoretical implications, it seemed advisable to present a preliminary report.

Methods and materials. Plasmapheresis technic. The donor lies on a table placed on a raised platform which, by gravity, increases the rate of flow of blood to the Fractionator which is about 40 inches lower. The phlebotomy is started with the insertion of a silicone-coated 15 gauge needle into a larger vein, usually in the antecubital fossa. The blood flows through a 3 way stop cock‖ and through an ion exchange resin column¶ which removes the calcium and some of the platelets. The decalcified blood then continues into the centrifuge bowl, which for this operation is designed to accept a fixed volume of 500 ml of fluid for each run. While the bowl is spinning, only the plasma (the lighter component) is allowed to flow from the bowl into a plastic bag. No admixture of red blood cells occurs. The red blood cells, which are heavier, accumulate within the bowl and flow by gravity, after the bowl is stopped, into another plastic bag. The plastic tubings, through which the plasma and red blood cells respectively flow to the bags and which are connected to the bowl are then sealed** (a one-half inch wide seal), and the seal is cut through the middle with a pair of scissors. This operation thus produces sterile containers of plasma and red blood cells with short lengths of attached tubing sealed at the end. Red blood cells are returned to the donor by connecting the plastic tubing attached to the red cell bag, to the 3 way stop cock. Incorporated in the plastic tubing outlet of the red cell bag is a drip chamber and a nylon mesh filter. In the 3 to 4 minute period during which the red cells drop from the centrifuge into the bag, sterile physiological saline is slowly infused into the donor in order to keep the lumen of the needle and tubing open for the return of the red blood cells. To some extent, before the red cells are returned, the saline replaces temporarily the volume of the whole blood removed.

Donors. Twenty-three men are being utilized in this study. Nineteen of these volunteers are members of the Philadelphia Park Police between the ages of 26 and 35, in excellent physical condition. The remaining 4 men are between 37 and 43 years of age. *Tests.* The following determinations are made at each visit: Blood pressure, pulse, red blood cell count, white blood cell count, differential counts, platelet count, hemoglobin, hematocrit, sedimentation rate and prothrombin clotting time. In addition, paper electrophoretic pattern and nitrogen content are determined for each plasma.

Results. Table I shows that there is very little variation in the red cell count, hemoglobin, hematocrit and plasma nitrogen in a single donor subjected to biweekly plasmapheresis over a period of 40 weeks. In addition the amount of time for each phlebotomy and red blood cell return is recorded. These data, pertaining to a single donor, are representative of the entire group of 23 individuals in the study.

White blood cell counts, differential counts,

† Mfd. by A. D. Little, Inc.

‡ Mfd. by Fenwal Labs., Inc.

§ Mfd. by General Electric.

‖ The 3 way stop cock is being replaced by a plastic Y tube.

¶ Dowex 50.

** Mfd. by Scientific Specialties Corp.

TABLE I. Effect of Biweekly Plasmapheresis on the Same Donor over a Period of 40 Weeks.

	Weeks										
	0	4	8	12	16	20	24	28	32	36	40
Red blood cell counts ($\times 10^6$)	4.7	4.9	4.6	4.5	5.6	5.2	4.9	5.4	5.1	4.7	4.9
Hemoglobin (g)	15.8	15.2	15.1	16.3	15.4	15.0	15.7	15.0	15.8	14.9	14.5
Hematocrit (%)	42	40	41	38	40	40	40	43	45	43	46
Plasma N (%)	1.07	1.14	1.00	1.02	1.08	1.00	1.03	1.01	1.04	1.08	1.06
Phlebotomy time*	7 35	9 45	9 00	7 40	7 46	10 02	7 21	7 40	15 10	7 23	7 40
R.B.C. return time*	7 50	10 10	9 12	12 35	9 40	7 05	8 30	9 08	11 12	10 57	9 34

* First number in these columns is minutes, last 2 numbers are seconds; *e.g.*, 7 35 = 7 min. 35 sec.

Figures on alternate biweekly plasmaphereses are omitted because of lack of space.

platelet counts, sedimentation rates and prothrombin clotting times carried out routinely at each donor's visit have been quite constant, much the same as would be found in any normal person.

To make this technic a practical procedure, the time consumed must be relatively short. The average time required for the phlebotomy and red blood cell return of the 23 men for 20 plasmaphereses (a total of 460) is 8′ 50″ and 10′ 04″ respectively. Allowing 3 minutes for the red cells to drop after the centrifuge has been stopped, the total time elapsed is 8′ 50″ + 10′ 04″ + 3′ = about 22 minutes. It is a simple matter to run 3 complete plasmaphereses per hour by beginning a phlebotomy on the second donor after the red blood cells are started in return to the first donor.

Over 550 plasmaphereses have been carried out since the start of this project including the preliminary and trial runs. No untoward reactions have been observed in any donor.

Discussion. The utilization of the new equipment mentioned has made frequent plasmapheresis of the same human donors both safe and feasible. This permits the use of donors for plasma only, with the replacement of their red blood cells, thus allowing 26 bleedings per year in this study. Fifty plasma donations yearly should be perfectly feasible since the human plasma proteins are regenerated rapidly(1,3), and since a very small percentage of the total plasma proteins is removed at one time. (200 ml plasma x 7.5% protein equals 15 g of protein out of a total plasma protein content of about 250 g, or about a 6% removal at one time.)

If 10 times the customary number of donations can be obtained from a single individual, it should be possible to achieve large savings in the collection of plasma since only one-tenth the number of donor centers would be required.

By plasmapheresis, single plasmas, as free of bacteria and hepatitis virus as the usual single donation of whole blood, can be preserved in non-porous bags, easily stacked and non-breakable, which can be left at any desired temperature for the inactivation of serum hepatitis virus. This virus is probably present in 0.5 to 1% of individual bleedings from the general population, but with selected donors for repeated plasmapheresis the incidence of virus in single plasmas should be considerably less than in whole blood. The possibility of untoward reaction in the recipient, to certain plasmas could be obviated by testing the individual plasma before use.

The plasmapheresis technic makes feasible the use of human donors in the preparation of high titered specific antibodies for therapy. The danger of foreign protein reaction is practically eliminated. Furthermore, it has been found that, unit for unit, the ratio of effectiveness of human antibody is far superior to horse antibody(8). As part of the current project the persistence of viral and bacterial antibodies in hyperimmune and other donors undergoing biweekly plasmapheresis is being determined. A further use of this method would be to obtain relatively large amounts of plasma from individuals who have rare antibodies for a short period of time, *e.g.* mothers following delivery, who have certain Rh or Hr antibodies which decrease in titer fairly rapidly.

Summary. Biweekly plasmapheresis on the

same human donors over a period of 40 weeks has been found to be feasible and safe. The possibilities for obtaining large amounts of concentrated human antibodies and for stockpiling sterile human plasmas are discussed.

1. Co Tui, F. C., Bartter, A. M., and Holt, R. B., *J. Am. Med. Assn.*, 1944, v124, 331.

2. Whipple, G. H., *Am. J. Med. Sci.*, 1938, v196, 609.

3. Elman, R., Charnas, R., and Davey, H. W., *Arch. Surg.*, 1943, v47, 216.

4. Adams, W. S., Blahd, W. H., and Bassett, S. H., PROC. SOC. EXP. BIOL. AND MED., 1952, v80, 377.

5. Davidson, C. S., and Gabuzda, C. J., Jr., personal communication.

6. Stokes, J., and Smolens, J., *Modern Problems in Pediatrics*, 1954, v1, 423.

7. Tullis, J. L., *et al.*, in preparation.

8. Ipsen, J., personal communication.

Received March 15, 1956. P.S.E.B.M., 1956, v91.

Failure of Ovarian Hormones to Maintain Pregnancy in Rats Deficient in Pantothenic or Pteroylglutamic Acid.* (22348)

MARJORIE M. NELSON AND HERBERT M. EVANS.

Institute of Experimental Biology, University of California, Berkeley.

Recent studies have shown that reproductive failure, *i.e.*, fetal death and resorption, can be prevented and pregnancy maintained by daily injections of estrone and progesterone in rats subjected to 4 different dietary deficiencies, those of thiamine(1), pyridoxine (2), protein(3,4) or potassium(5). The daily dosage levels of the hormones were the same as those found to be effective in maintaining pregnancy in rats hypophysectomized and oophorectomized after breeding(6). The present communication reports the failure of these hormones to prevent fetal death and resorption in rats deficient in either pantothenic acid(7) or pteroylglutamic acid(8,9).

Methods. The procedures for testing the efficacy of the hormones were the same as those used previously to maintain pregnancy in other dietary deficiencies(1-5). The experimental conditions necessary for each vitamin deficiency to result in fetal death and resorption in approximately 90% of the animals were determined, if not already known. The synthetic hormones were dissolved in sesame oil and given daily, either separately or in combination, by subcutaneous injection from the 3rd or 5th days of gestation period to the day before autopsy. Levels of estrone varying from 0.5 to 2.0 μg† daily were given separately and levels from 0.5 to 1.0 μg were given in combination with 4 mg of progesterone. Five to ten rats (Long-Evans strain) were used for each experimental group. Vaginal smears were examined daily for the presence of erythrocytes, the sign of implantation. The rats were autopsied near the end of the gestation period, from the 15th to the 21st day. The basal (control) purified diet was composed of 24% alcohol-extracted casein, 64% sucrose, 8% hydrogenated vegetable oil (Crisco or Primex), and 4% salts no. 4(10). Crystalline vitamins per kilo of diet were: 300 μg d-biotin, 5 mg 2-methyl-1,4-naphthoquinone, 5 mg thiamine HCl, 5 mg pyridoxine HCl, 5.5 mg pteroylglutamic acid, 10 mg riboflavin, 10 mg p-aminobenzoic acid, 20 mg

* Aided by grants from Roche Anniversary Foundation and the U. S. Public Health Service A-470 and A-841. We are indebted to Dr. Daniel A. McGinty of Parke, Davis and Co., Detroit, Mich. for generous supplies of estrone and progesterone; to Dr. T. H. Jukes, Lederle Laboratories, Pearl River, N. Y. for pteroylglutamic acid; to Dr. Elmer L. Severinghaus of Hoffmann-LaRoche, Nutley, N. J. for d-biotin, d-calcium pantothenate and dl-alpha-tocopherol; to Dr. Richard H. Barnes of Sharp and Dohme, Glenolden, Pa., for succinylsulfathiazole (SST); and to Dr. Randolph Major, Merck and Co., Rahway, N. J. for crystalline B vitamins and ?-methyl-1,4-naphthoquinone.

† Maintenance of pregnancy has been shown to vary with level of estrone given to protein-deficient rats(3).