that phenylketonurics serum serotonin levels were significantly higher after ingestion of a low-phenylalanine diet for 4 weeks than before dietary control. Their failure to note significant differences in excretion of 5-hydroxyindoleacetic acid in the time studied, was attributed to use of random rather than 24-hour samples of urine. Ages of patients studied were not given. While our studies were in progress, Berendes *et al.*(7) reported that phenylketonurics who had been fed low-phenylalanine diets excreted more 5-hydroxyindoleacetic acid/24 hours than did untreated phenylketonurics. Creatinine values were not given.

Summary. 1. Following a decrease in serum phenylalanine levels (induced by feeding of a low-phenylalanine diet) phenylketonurics excrete increased amounts of 5-hydroxyindoleacetic acid. 2. The ratio of urinary 5-hydroxyindoleacetic acid to creatinine is about 5-fold greater for young phenylketonuric children, fed a low-phenylalanine diet for 4-10 months, than for untreated phenylketonurics. 3. Oral administration of L-tryptophan to untreated phenylketonuric was followed by increase in 5-hydroxyindoleacetic acid excretion. 4. Following oral administration of 5-hydroxytryptophan, 10-15% was excreted as urinary 5-hydroxyindoleacetic acid by an otherwise untreated phenylketonuric.

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Production of Leucocytic Exudates in Rat Granuloma Pouch.* (24687)

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A relatively simple procedure for producing inflammatory exudates rich in leucocytes, based on rat granuloma pouch technic of Selye *et al.*(1,2,3) is reported in this paper. Several technics for production of sterile inflammatory exudates have been reported. Opie(4,5,6,7) used aleuronat and starch for production of sterile exudates in the pleural and peritoneal cavities of dogs; Ponder and MacLeod(8) employed intraperitoneal saline injection method in rabbits. Menkin(9) employed turpentine injections for production of sterile pleural exudates in dogs. Technics for separation of leucocytes from whole blood have been reported by Tullis(10). The granuloma pouch technic as developed by Selye and coworkers for study of anti-inflammatory agents(3) appeared to us to offer several advantages for the purposes of our studies. Conditions necessary for producing an inflammatory exudate rich in myeloperoxidase-containing polymorphonuclear leucocytes are here reported.

Methods. Male, Sprague-Dawley rats were used. *Formation of pouch.* After ether anesthesia, the back of the animal was shaved with fine electric clippers and 1% iodine solution applied on the skin. Forty to 60 ml of air were introduced subcutaneously by means of a syringe and 22 gauge needle. The pouch usually covered about ¾ of the back of the

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animal. Care must be taken at time of insufflation to prevent air from going into the forelegs and cervical region. This can be avoided by putting pressure on the skin with the free hand. The irritant was introduced immediately after the pouch was formed. *Collection and nature of cells in exudate.* The fluid formed in the pouch was extracted with a syringe attached to 22 gauge hypodermic needle. The collected exudate was centrifuged at room temperature and leucocytes washed 3 times with 0.9% saline. Wright stain(11) and benzidine stain(12) were used to differentiate the cells and to evaluate the myeloperoxidase content. Cell counts were made with hemocytometer(13). *The following aspects were evaluated*: a) size of pouch, b) regularity and shape of the pouch, c) volume of exudate, d) nature of exudate, e) number of leucocytes, f) per cent neutrophils, g) myeloperoxidase content. Various factors that might affect cellular composition of the exudate were also studied. The pouch was always formed in the same general way and only one of the factors was varied in a group of rats at any given time. Groups were from 5 to 10 animals. The factors investigated were 1) irritant used, 2) number and type of injections of turpentine, 3) size of animal, 4) local pH and 5) time of withdrawal of fluid.

Results. Although there was some individual variation the findings with each group are sufficiently reproducible to permit evaluation of the factors under consideration.

1) *Effect of irritant used.* a) Croton oil at 0.5% (v/v) concentration in corn oil consistently produced hemorrhagic exudates with relatively few leucocytes of low myeloperoxidase content. b) Peptone culture broth produced no significant amount of exudate. c) Turpentine (commercial preparation) gave good results. The volume of exudate varied from 4 to 10 ml and the purulent fluid contained 60 to 80% neutrophils with high myeloperoxidase activity.

2) *Number and type of injections of turpentine.* Three-tenths ml of turpentine was introduced on first and fifth day, and fluid withdrawn on seventh day. When only one injection of turpentine was used no fluid was detected in the pouch. A second injection on the day previous to withdrawal was effective in producing the exudate. When this second injection was applied between fourth and sixth day after first injection, consistently good results were obtained, but when it was applied after the sixth day, only a small amount of exudate was found (1 to 3 ml) and percentage of neutrophils and myeloperoxidase content were low.

In groups given single injections of 0.5 ml of 5%, 10% and 50% turpentine solution in corn oil, the amount of exudate was small. Percentage of neutrophils was high but myeloperoxidase activity was low.

One group injected with 0.3 ml of turpentine alone showed good results, but the pouches were quite irregular. The best results were obtained when rats were injected with 10 ml of 3% (v/v) suspension of turpentine in 0.01 M phosphate buffer pH 7.0. One percent solution of Alconox was added (1 ml to each 9 ml suspension) and the suspension made homogeneous by shaking immediately before injection. A suspension of Alconox and buffer without turpentine had no effect.

3) *Effect of size of rats.* Rats over 250 g were not found suitable for formation of regular pouches. Best results were observed with groups of rats weighing between 150-200 g. In a group of small rats (100-125 g) 7 out of 10 died on day the pouch was formed. Good results were obtained in survivors. 4) *Effect of local pH.* (Table I) Five groups of rats received turpentine suspended in 0.01 M phosphate buffer at pH's of 6.0, 6.5, 7.0, 7.5 and 8, in groups 1 to 5 respectively. Three out of 10 animals died in the first group and 6 out of 10 in the fifth. No difference was observed in nature of exudate formed in rats in Groups 2, 3, 4 and survivors in Groups 1 and 5.

5) *Effect of time of withdrawal of fluid.* When the exudate is obtained 24 hours after second injection of turpentine, the percentage of neutrophils is high (90-100%). After 48 hours the percentage of leucocytes was 70-90 and when 3 days elapsed after second injection the neutrophil percentage dropped to 50-

TABLE I. Effect of pH of Turpentine Suspension on Leucocyte Production, in 5 Groups.

No. of rats	pH of turpentine suspension	Nature of exudate	Vol (ml) Range	Vol (ml) Avg	No. of cells $\times 10^{-3}$ Range	No. of cells $\times 10^{-3}$ Avg	% neutrophils	MPO content	pH of exudate
10*	6.0	7 purulent	4-7	5	15-25	18	90-100	3+ to 4+	
9	6.5	8 purulent 1 hemorrhagic	5-9	6	10-28	20	90-100	*Idem*	7.0 to 7.2
7	7.0	7 purulent	3-9	6	12-22	16	90-100	"	
8	7.5	7 purulent 1 hemorrhagic	5-8	5.5	15-25	19	90-100	"	7.0 to 7.2
10†	8.0	4 purulent	4-8	5	9-23	14	90-100	2+ to 4+	

* Three animals died in Group 1. † Six animals died in Group 5.

Animals inj. with 3% turpentine suspension in 0.01 M phosphate buffer pH as indicated in column 2, plus Alconox. First inj. made immediately after pouch was formed; second inj. made 5 days later. Exudate was collected on day following second inj.

60. Repeated injections of turpentine suspension gave satisfactory yields of leucocytes. Thus, if a second injection is made on fifth day after pouch is formed, and the exudate collected on sixth day, a third injection (20-30 ml of turpentine suspension) on ninth day resulted in a good yield on the tenth day. As many as 5 injections have been made in groups of rats with good yield of exudate resulting on day following. With successive injections of turpentine suspensions, at pH 7.0, it is possible to realize an exudate of 5-10 ml/rat, each time with a count ranging from 10-30 x 10^3 leucocytes mm^3, and a high neutrophil percentage (90-100). The myeloperoxidase content was consistently high in these exudates. Turpentine suspensions older than 2 weeks turn yellow and are highly toxic. The suspension should be stored in a brown bottle in the cold and prepared fresh each week. The exudate usually has a layer of turpentine floating on top after centrifugation, which is readily removed by decantation.

Evolution of the pouch. The first injection of the irritant causes formation of a granulomatous membrane apparent by the fourth day. The second injection of the irritant initiates appearance of exudate in the pouch. By seventh day the pouch begins to shrink and becomes quite small by sixteenth to seventeenth day. By the twentieth to twenty-second day the pouch collapses leaving a fibrous scar. In most animals a small necrotic area of skin is observed. There is, however, complete recovery, and it is not possible to distinguish treated animals after 5 or 6 weeks from normal animals.

Discussion. The granuloma pouch technic presented has proved most satisfactory for producing an exudate which contains relatively large quantities of myeloperoxidase-rich leucocytes in the rat. Gross contamination with erythrocytes was only occasionally experienced.

No difference in effect of local pH as far as cellular composition of exudate is concerned was observed. Control of this factor, however, was limited to adjustment of pH of the turpentine suspension. This criterion has questionable validity, however, since the pH of exudates was between 7.0 to 7.2, when pH of the injected suspension varied between 6.5-7.5. How soon the adjustment of pH is effected inside the pouch is not known. We were interested in the effect of this factor from reports of Menkin *et al.*(9,14,15,16) who state that pH has a marked influence on cellular composition of the exudate with local acidosis favoring disappearance of polymorphonuclear leucocytes. Harris(17) has challenged this view, stating that from present evidence no connection between pH and composition of inflammatory exudates has been established. Menkin(18) has answered Harris' criticism by stating that the different results might be due to differences in material under study, since leucocytes from several animal species behave differently in several aspects. Studies in rabbits by Harris(19) failed to show any correlation between pH and cellular sequence of the exudate. The guinea pig

seemed to show a slightly better correlation (20). To our knowledge systematic studies on this aspect have not been done with the rat.

Another controversy has arisen concerning use of turpentine to produce leucocytic exudates. Harris(19) reported it to be toxic to cells. Menkin(18) explains his different observations as due to use of different species. We can only say that in our experiments the leucocytes in the exudate which results from injection of turpentine in the granuloma pouch showed high myeloperoxidase activity after being washed with saline.

Summary. The rat granuloma pouch has been found to be a convenient source for collection of leucocytes rich in myeloperoxidase. Of a number of irritants tried, a suspension of turpentine was most satisfactory. Within a limited range, pH of turpentine suspension had no significant effect on cellular composition of the exudate.

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Effects of Dietary Fat upon Polyunsaturated Fatty Acids of Blood in Patients with Multiple Sclerosis.*† (24688)

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Among several hypotheses advanced concerning the etiology of multiple sclerosis (MS), 2 schools of thought have recently gained prominence; one(1) proposes that an allergic reaction is involved; the other(2,3) that an enzymatic or metabolic defect underlies recurrent attacks of demyelination. We reported previously that some patients with MS revealed higher plasma levels of dienoic acid than did controls(4). This observation, together with the dietary studies of Swank(5) and increased chylomicron index noted by Skillen, *et al.*(6), warrants further investigation of the lipid metabolism in this disease.

Methods. The plan of this study was similar to that of Holman, *et al.*(7). Diagnosis of MS was established as outlined previously (4). A baseline blood sample was obtained after patient had been on average hospital diet including approximately 20% protein, 28% fat, and 52% carbohydrate. Then, all individuals received a nearly eucaloric diet with soya bean oil as main source of polyunsaturated fatty acids (PUFA). Composition of diet, its lipids and vitamins added, is given in Tables I-A, B and C. Diagnoses of control patients are listed in Table II. Sequence of

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