GRANULOCYTOPOIETIC FRACTION OF YELLOW BONE MARROW

THESIS

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for the Degree of Doctor of Philosophy

By

Joseph Zichis

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INTRODUCTION

In recent years the condition known as granulocytopenia has attained a preminent position in the medical world. While the etiology of the disease still remains obscure, the many agents employed for its treatment have met with varying degrees of success. (1,2,3). Of these therapeutic agents, yellow bone marrow has attracted much attention.

Watkins (4) has obtained encouraging results by oral administration of bone marrow at the onset of the disease. He gave daily doses of approximately 60 to 120 gm. of fresh yellow bone marrow, obtained from beef, until the blood assumed a normal picture, and smaller doses for several months later. Since large amounts of marrow were necessary to obtain a change in the leukocytic picture, and since in certain cases the patient was unable to digest and assimilate such large quantities, the use of the fresh marrow for this purpose was considered undesirable. Accordingly, a study was undertaken to separate the granulocytopoietic fraction from yellow bone marrow, and to determine its activity on experimental animals.

EXPERIMENTAL

SOURCE OF BONE MARROW - The bone marrow which was used in these studies was obtained from fresh long bones of cattle. It was freed of blood, bony and fibrous material and ground.

PART I

METHOD OF PREPARING CONCENTRATES

EXTRACTIONS 1 to 10 --- 10 Kgm. of bone marrow was dehydrated by thoroughly mixing it with 10 Kgm. of plaster of Paris. The mixture was allowed to dry over-night, and then broken up into small particles. 2 Kgm. of this mixture was placed into each of 10 percolators and extracted at room temperature with the following solvents:

1) Acetone alcohol (10% acetone)
2) Acetone
3) Chloroform
4) 95% Alcohol
5) Absolute methyl alcohol
6) Absolute ethyl alcohol
7) Dichloroethylene
8) Ether
9) Carbon tetrachloride
10) Petroleum ether

Each of the above solvents dissolved such a large amount of fat from the bone marrow that it was found impossible to determine their hematopoietic properties. Due to the lack of concentration this method was considered useless and discarded.

PREPARATION 1, (P-1) --- 2 Kgm. of bone marrow was saponified by boiling with 4 liters of 3% alcoholic potassium hydroxide and 4

liters of 95% alcohol for 30 minutes under a reflux condenser. When the saponification was complete and while the solution was still hot, it was filtered through a large fluted filter. The residue consisted mostly of fibrous and bony structures. To the filtrate 3 liters of distilled water was added, and heated to boiling. The solution was cooled, and 1 liter portions were extracted by shaking thoroughly with 400 cc. of petroleum ether (B.P. 40° - 70° C.). In order to prevent the formation of an emulsion 50 cc. of distilled water was added to each 1 liter portion. After the separation of the petroleum ether layer the underlying soap solution was drawn off. The ether solution was washed 5 times with 50 cc. of 40% alcohol. When all the saponifying alcoholic solution was thus extracted the petroleum ether solution was collected and the ether removed by heating over a steam-bath. A yield of 4.8 gm. of the nonsaponifiable material was obtained.

The substance was a dark yellow, oily liquid, having a slight acrid odor and an oily after-taste. The specific gravity was .8940 and it gave a negative test for sterols. The substance was soluble in 95% alcohol, ether, petroleum ether and chloroform. When administered orally to animals in which a condition of granulocytopenia had been produced, it showed no granulocytopoietic activity.

The results of the biological test are given in Table I.

PREPARATION 2. (P-2) --- 2 Kgm. of bone marrow was saponified with 6 liters of 3% alcoholic potassium hydroxide at room temperature for 24 hours. The saponification was aided by mixing the solution with a mechnical stirrer. At the end of 24 hours the solution was filtered through a large fluted filter. The residue consisted chiefly

of insoluble bony and fibrous tissues. An equal volume of distilled water was added to the filtrate which separated into two layers. The upper layer containing the nonsaponifiable fraction was saved and the lower soapy layer discarded. In order to free the concentrate from alkali it was washed 5 times with 50 cc. of distilled water and 5 times with 50 cc. of 40% alcohol. From the original 2 Kgm. of bone marrow, 100 gm. of the concentrate was obtained.

The product thus obtained was a yellow, oily liquid, being nearly odorless and having a slightly oily after-taste. It had a neutral reaction to phenolphthalein; gave a negative test for sterols and had a specific gravity of .8626. It was soluble in 95% alcohol, ether, petroleum ether, chloroform, and only slightly soluble in methyl alcohol. This concentrate when administered orally to animals having the granulocytopenic condition showed marked granulocytopoietic activity.

The results are shown in Table 3.

PREPARATION 3, (P-3) --- 2 Kgm. of bone marrow was saponified and filtered in exactly the same manner as under Preparation 2. 1 liter of distilled water was added to the filtrate. After shaking thoroughly, 1 liter portions of the filtrate were extracted with 500 cc. of petroleum ether (B.P. 40°-50°C.). After the ether layer separated out the underlying soap solution was drawn off. When all the filtrate was thus extracted, the petroleum ether solution was resaponified with 3 liters of 3% alcoholic potassium hydroxide at room temperature for 24 hours. The soap formed by this procedure was insoluble in the liquid medium and was easily separated by filtration aided by suction. The soap was washed 3 times with 50 cc. portions of petroleum ether. The addition of 1 liter of distilled

water to the filtrate caused the formation of 2 layers. The upper layer was saved and the lower discarded. 1 liter portions of the petroleum ether solution were washed 5 times with 100 cc. of 40% alcohol. When all of the ether solution was thus washed, it was collected and distilled off under diminished pressure at 30°C. The resulting product had a slight cloudiness, but after filtering through a layer of asbestos it remained clear. 20 gm. of the concentrate was obtained from the original 2 Kgms. of bone marrow.

This concentrate was a brilliant amber, oily liquid, being odorless and imparting a faint oily taste. The specific gravity was .8276. It gave a neutral reaction to phenolphthalein and a negative test for sterols, and it was soluble in 95% alcohol, ether, petroleum ether and chloroform. When this substance was administered intramuscularly to animals which showed a condition of gramulocytopenia, a marked granulocytopoietic activity was observed within 4 hours.

The results are given in Tables 5 and 6.

PART II

ETIOLOGY OF GRANULOCYTOPENIA --- Since the disease granulocytopenia was first described in Germany in 1922 (5) and in the United States in 1924 (6), many efforts (6,7,8,9,10,11,12,13,14,15,) have been made to determine its cause. Many theories of the etiology of this disease have been advanced, namely, dietary deficiency (7,8), hormonic dysfunction (9,10), use of certain coal-tar derivatives (11,12,13), and bacterial infection (6,14,15).

The first theory is supported by the work of Langston and Day (7) who were able to demonstrate profound leukopenia in monkeys by feeding them a diet deficient in Vitamin C. However, these animals also showed deficiencies in red blood cells and blood platelets. In addition, Miller and Rhoads (8) produced a similar blood picture by feeding dogs a diet used to produce black tongue.

The second theory is upheld by the studies of Britten and Corey (9) who demonstrated leukopenic conditions in cats which were adrenalectomized. Others also (10,11,12) are of the opinion that the condition may be associated with some hormonic dysfunction, because in many instances the onset of the disease occurred during the menstrual period.

Weiskotten (13) supports the chemical etiology by showing that the administration of small doses of benzene to rabbits caused a decided leukopenic condition. Furthermore, Krueke (14) by injecting intravenously small doses of benzene into rabbits produced profound granulocytopenia, and from this he concluded that benzene has a selective action on the leukopoietic system. This theory is also upheld by clinicians (15,16,17) who claim that the disease frequently develops as a result of the use of certain coal-tar derivatives.

The bacterial etiology was first demonstrated by Lovett (5) who produced leukopenic conditions in guinea-pigs by injecting cultures of Bacillus pyocyaneus intraperitoneally. In addition, Dennis (13) found that Staphylococcus aureus or Streptococcus hemolyticus, when placed into a rabbit in such a manner as to liberate toxins by dialysis, without causing a general bacterial infection, developed a severe type of leukopenia. More recently, Felsen (19) described 3 cases of acute bacillary dysentery infection which terminated in the production of profound granulocytopenia. Moreover, Sholl (20) described a case of granulocytopenia in a pig which upon autopsy showed a severely infected gall bladder.

METHODS OF PRODUCING GRANULOCYTOPENIC CONDITIONS EXPERIMENTALLY

ANIMALS — Healthy, full-grown rabbits and rats were used and each sex was represented in about equal numbers. The animals were kept on a normal diet. Blood was obtained from the marginal vein of the ear in the case of the rabbits, and from the tail in the case of rats. The blood smears were stained with Wright's Stain.

1. CHEMICAL METHODS

(a) BENZENE — .1 cc. of benzene was administered orally to each of 24 rats and 1 cc. to each of 8 rabbits daily for 10 weeks. Another group of 8 rabbits received daily intravenous injections of .05 cc. of benzene into each for 5 weeks.

The activity of the benzene on the leukopoietic system was determined in the rabbits by making daily total and differential leukocytic counts, and in the rats by making weekly counts.

The results of this study did not show any changes in the blood picture, and for this reason only the average values are given as follows:

1.	Total leukocytes	Rabbits	8500,	Rats	14500
2.	Polymuclear neutrophiles	"	52%	"	19%
3.	Monocytes	"	1%	"	1%
4.	Transitional cells	"	2%	"	3%
5.	Eosinophiles	"	2%	"	2%
6.	Basophiles	"	3%	"	-
7.	Small lymphocytes	"	34%	"	50%
8.	Large lymphocytes	"	6%	"	25%

(b) AMIDOPYRINE -- Daily doses of a 100 mgm. of amidopyrine per Kgm. of body-weight were administered subcutaneously to each of 12 rabbits for 12 weeks.

The leukopoietic activity of this compound was determined by obtaining total and differential leukocytic counts daily for the first 3 weeks and weekly during the remaining time.

The results demonstrated no changes in the blood picture. The average values are as follows:

1.	Total leukocytes	8900
2.	Polymuclear neutrophiles	49%
3.	Monocytes	1.5%
4.	Transitional cells	.5%
5.	Eosinophiles	2%
6.	Basophiles	2%
7.	Small lymphocytes	36%
8.	Large lymphocytes	9%

(c) HYDROQUINONE --- 25 mgm. of hydroquinone was administered intravenously to each of 2 rabbits. The animals were males and each weighed approximately 2 Kgm. In addition 20 rats, (10 males and 10 females) weighing about 200 gm. each, were kept for 6 months on a stock diet containing 2% hydroquinone.

Total and differential leukocytic counts were made in order to determine the effect of the substance. In the rabbits daily counts were made and in the rats weekly counts for the first six weeks and thereafter once every 2 weeks.

On the 6th day of the experiment one rabbit died without showing any changes in the blood picture, whereas the other did not show any ill effects after 20 days. The blood counts on the rats did not show any changes. For this reason, only the average values are given.

	Rats	Rabbits
1. Total leukocytes	15000	8386
2. Polymuclear neutrophiles	20%	51%
3. Monocytes	1%	2.6%
4. Transitional Cells	2%	1.6%
5. Eosinophiles	1%	4%
6. Basophiles	-	1%
7. Small lymphocytes	50%	32%
8. Large lymphocytes	26%	8%

2. BACTERIAL METHOD --- The procedure used was a modification of the method described by Dennis (18). A #000 gelatin capsule coated with collodion was filled with a 24-hour culture of Staphylococcus aureus by means of a hypodermic needle and syringe. The capsule was sealed with a drop of collodion, and sterilised by immersing in a 1% solution of HgCl2 3 times at 5-minute intervals.

Later this procedure was modified by replacing the collodion coated gelatin capsules with Naturalamb skin sacs and by introducing a larger volume of the organism. 10 cc. of a 24-hour culture of the organism was placed into the sac, the open end of which was sealed by tying with a linen thread. The sac was sterilized by immersing several times in 70% alcohol.

The normal total and differential leukocytic counts were determined on each animal. The rabbits were starved for 20 hours before the operation. A light ether anesthesia was administered to the animals, and the peritoneal cavity was exposed by incision, care being exercised to maintain aseptic conditions. 3 capsules or 1 Naturalamb skin sac were placed into the lower part of the peritoneal cavity. The peritoneum and abdominal wall were sutured with plain cat gut. Metal clips were used on the skin. A bandage was applied and held in position by a binder.

The granulocytopoietic effect obtained by this procedure was determined by making daily total and differential leukocytic counts. The results of the total and differential leukocytic counts were used as the criterion of the effects produced on the granulocytopoietic system by the bone marrow concentrates.

46 rabbits were prepared according to this method. Of this number 17 developed a condition of granulocytopenia, 9 died without showing any change in the blood picture, and 20 recovered without showing any ill effects. The 17 animals that developed the granulocytopenic condition were used in the following experiments.

RABBITS 1 and 2 -- Female rabbits each weighing 3 Kgm. were prepared by placing 3 collodion coated capsules filled with a 24-hour culture of Staphylococcus aureus into each animal as described. The rabbits developed the granulocytopenic condition, and although 10 cc. of P-1 was administered by means of a stomach tube both animals died.

The data are given in Table 1.

TABLE I

RESULTS OF P-1

Rabbits	Dates	Condition	Substance Administered	Total Leukocytes	Polymuclear Neutrophiles	Polymuclear Neutrophiles, Degenerated	Monocytes	Transitional Cells	Basophiles	Eosinophiles	Small Lymphocytes	Large Lymphocytes
1	3/4-35	*N	-	8200	51	-	3	1	1	-	36	8
"	3/4-35	O	-	-	-	-	-	-	-	-	-	-
"	3/5-35	PO	-	5100	20	6	1	3	-	-	58	12
"	3/6-35	"	10 cc.P-1	3900	5	7	2	-	-	-	78	8
		D										
2	3/4-35	N	-	6800	39	-	4	-	2	1	43	11
"	3/4-35	O	-	-	-	-	-	-	-	-	-	-
"	3/5-35	PO	-	11,200	50	6	3	1	-	1	30	9
"	3/6-35	"	-	9000	12	20	6	-	-	-	49	13
"	3/7-35	"	10 cc.P-1	9500	6	-	2	3	-	-	84	5
		D										

* Note - N = Normal. O = Operated. PO = Post Operative. D = Died.

NOTE --- In these Tables the cells termed as Polynuclear Neutrophiles, Degenerated, were polynuclear neutrophiles characterized by disintegration of the cell membrane and cytoplasm (presence of vacuoles) and a distortion of the nucleus. These cells stained very poorly.

RABBITS 3,4,5 and 6 --- Rabbits 3 and 4 (males) and 5 and 6 (females) each weighing approximately 2.5 Kgm. were prepared in the same manner as the first 2. Each animal developed the granulocytopenic condition. 10 gm. of fresh yellow bone marrow was melted at 40°C., strained through silkcline and was administered to each animal by means of a stomach tube. Rabbits 3,4 and 6 died on the following day without showing any signs of recovery. Rabbit 5 received 3 doses of the melted marrow, but showed no recovery, and died on the 4th day.

The data are given in Table 2.

TABLE 2

RESULTS OF MELTED BONE MARROW

Rabbits	Dates	Condition	Substance Administered	Total Leukocytes	Polynuclear Neutrophiles	Polynuclear Neutrophiles, Degenerated	Monocytes	Transitional Cells	Basophiles	Eosinophiles	Small Lymphocytes	Large Lymphocytes
3	5/13-35	*N	-	8350	59	-	2	1	1	-	34	3
"	5/14-35	O	-	-	-	-	-	-	-	-	-	-
"	5/14-35	PO	-	5300	9	14	6	2	1	1	53	14
"	5/15-35	"	10 cc. MM	3000	3	6	2	1	-	-	78	10
"	5/17-35	" D	-	2050	2	-	-	-	-	-	90	8
4	5/13-35	N	-	6060	23	-	2	2	4	-	65	4
"	5/14-35	O	-	-	-	-	-	-	-	-	-	-
"	5/15-35	PO	-	5400	24	-	4	2	2	-	60	8
"	5/16-35	"	10 cc.MM	2600	3	11	3	-	-	-	73	10
"	5/17-35	D										
5	5/6-35	N	-	9050	34	-	5	2	2	-	55	2
"	5/6-35 p.m.	O	-	-	-	-	-	-	-	-	-	-
"	5/7-35	PO	10 cc MM	4500	9	3	2	-	-	-	73	13
"	5/8-35	"	"	4100	3	7	4	-	-	-	80	6
"	5/9-35	" D	"	2980	1	13	-	3	-	-	70	13
6	5/6-35	N	-	8250	53	-	3	1	1	-	38	4
"	5/7-35	N	-	8000	50	-	1	-	2	1	39	7
"	5/8-35	O	-	-	-	-	-	-	-	-	-	-
"	5/9-35	PO	-	5400	18	4	1	3	-	-	64	10
"	5/10-35	"	10 cc MM	2000	1	3	2	-	-	-	88	6
"	5/11-35	D										

*Note:- N = Normal. O = Operated. PO = Post Operative. D = Died.

MM = Melted Bone Marrow.

RABBITS 7 and 8 --- Male rabbits, each weighing 3 Kgm., were prepared according to the same procedure. Each animal developed a condition of granulocytopenia, and died before therapy could be instituted.

The data are shown in Table 3.

TABLE 3

RESULTS OF RABBIT THAT DIED BEFORE THERAPY COULD BE INSTITUTED

Rabbits	Dates	Condition	Substance Administered	Total Leukocytes	Polymuclear Neutrophiles	Polymuclear Neutrophiles, Degenerated	Monocytes	Transitional Cells	Basophiles	Eosinophiles	Small Lymphocytes	Large Lymphocytes
7	5/7-35	* N	-	9500	52	-	2	1	1	-	36	8
"	5/8-35	N	-	8900	50	-	3	2	4	1	35	5
"	5/9-35	O	-	-	-	-	-	-	-	-	-	-
"	5/10-35	PO	-	4000	1	9	4	-	-	-	76	10
"	5/11-35	D										
8	10/2-35	N	-	8500	50	-	2	1	-	-	39	8
"	10/2-35 p.m.	O	-	-	-	-	-	-	-	-	-	-
"	10/3-35	PO	-	14400	80	9	1	-	-	-	8	2
"	10/4-35	"	-	4560	10	11	3	-	-	-	69	7
"	10/5-35	D										

*Note: N = Normal. O = Operated. PO = Post Operative. D = Died.

RABBITS 9, 10, 11 and 12 — Rabbits 9 and 10 (males) and 11 and 12 (females) each weighing about 2.5 Kgm. were prepared as the preceding ones. All developed the granulocytopenic condition and recovered upon daily oral administration of P-2.

The data are given in Table 4.

TABLE 4

RESULTS ON P-2

Rabbits	Dates	Condition	Substance Administered	Total Leukocytes	Polymuclear Neutrophiles	Polymuclear Neutrophiles, Degenerated	Monocytes	Transitional Cells	Basophiles	Eosinophiles	Small Lymphocytes	Large Lymphocytes
9	4/9-35	* N	-	8700	52	-	2	2	1	-	38	5
"	4/9-35	O	-	-	-	-	-	-	-	-	-	-
"	4/10-35	PO	-	5100	28	2	2	-	-	-	60	8
"	4/11-35	"	5 cc.P-2	2900	20	-	3	1	-	-	67	9
"	4/12-35	"	-	8900	50	-	4	2	1	-	37	6
"	4/13-35	"	5 cc.P-2	9150	8	2	1	-	1	-	80	8
"	4/15-35	"	"	8500	45	-	2	-	-	1	45	7
"	4/16-35	"	"	9700	51	-	3	1	2	-	33	10
"	4/17-35	"	"	9000	50	-	4	-	-	-	40	6
		D										
10	4/9-35	N	-	8750	47	-	6	1	1	-	32	13
"	4/9-35	O	-	-	-	-	-	-	-	-	-	-
"	4/10-35	PO	-	7200	16	-	-	1	1	-	71	11
"	4/11-35	"	5 cc.P-2	8750	16	-	3	-	1	1	70	9
"	4/12-35	"	"	15500	57	-	-	3	-	-	28	12
"	4/13-35	"	"	9050	48	-	4	-	1	-	39	8
"	4/15-35	"	"	9000	50	-	3	2	2	1	36	6

TABLE 4 - Continued

Rabbits	Dates	Condition	Substance Administered	Total Leukocytes	Polynuclear Neutrophiles	Polynuclear Neutrophiles Degenerated	Monocytes	Transitional Cells	Basophiles	Eosinophiles	Small Lymphocytes	Large Lymphocytes
11	4/9-35	* N	-	8700	46	-	2	1	-	1	40	10
"	4/10-35	O	-	-	-	-	-	-	-	-	-	-
"	4/12-35	PO	-	10000	30	8	1	-	1	-	45	15
"	4/13-35	"	-	9050	33	3	4	-	-	-	53	7
"	4/13-35	"	5 cc.P-2	5100	7	-	1	-	-	-	82	10
"	4/15-35	"	"	8900	40	5	-	3	1	1	41	9
"	4/16-35	"	"	7500	51	-	3	1	-	1	34	10
"	4/17-35	"	"	9000	56	-	1	2	1	-	27	13
"	4/18-35	"	"	9300	49	-	5	-	1	1	40	4
		D										
12	5/6-35	N	-	9350	45	-	1	3	2	-	46	3
"	5/7-35	N	-	9950	50	-	3	1	1	-	42	3
"	5/8-35	O	-	-	-	-	-	-	-	-	-	-
"	5/9-35	PO	-	4050	17	9	1	-	-	-	65	8
"	5/10-35	"	10 cc.P-2	2500	4	10	2	3	-	-	70	11
"	5/10-35 p.m.	"	-	6000	40	2	4	1	1	-	45	7
"	5/11-35	"	10 cc.P-2	4550	19	-	1	3	2	-	59	16
"	5/13-35	"	"	8500	47	-	2	-	-	-	41	10
"	5/14-35	"	"	9000	52	-	1	1	1	-	40	5
"	5/15-35	"	"	7050	48	-	3	-	-	1	38	10
"	5/17-35	"	"	8600	50	-	6	-	-	1	37	6
		D										

Note: N= Normal. O = Operated. PO = Post Operative. D = Discontinued

OBSERVATION ON THE ABSORPTION AND LOCAL REACTION of P-3 ---- 12 full grown rats and 3 full grown rabbits were used. The right leg of each animal was shaved and the skin was cleaned thoroughly with soap and water. Under aseptic conditions .2 cc. of P-3 was injected into the muscle of the leg daily for 4 days. Each injection was made at the same site.

The injections did not cause any local reaction as manifested by the absence of redness, swelling and restlessness in the animals. 4 days after the last injections were made the animals were killed and upon autopsy the muscles into which the preparation was injected showed no gross abnormality. This indicates definitely that P-3 is easily absorbed when injected into an animal intramuscularly.

RABBITS 13, 14 and 15 ---- Female rabbits, each weighing approximately 2.5 Kgm., were prepared according to the method described, and a Naturalamb skin sac containing 10 cc. of a 24-hour culture of Staphylococcus aureus was placed into the peritoneal cavity of each animal. Rabbit 13 did not develop a typical condition of granulocytopenia. The second day after the operation it had a total leukocyte count of 31000, and normal polymuclear neutrophiles could not be found. The animal was given .1cc. of P-3 intramuscularly daily for 6 days. At the end of this period, the blood picture returned to normal and the rabbit showed apparent recovery. 48 hours after the operation rabbits 14 and 15 showed a condition of granulocytopenia. Rabbit 14 was given .5 cc. and Rabbit 15, .1 cc. of P-3 intramuscularly daily for 3 days. At the end of the 3-day period both animals showed a normal blood picture and both appeared to be in good health.

The data are shown in Table 5.

TABLE 5

RESULTS ON P-3

Rabbits	Dates	Condition	Substance Administered	Total Leukocytes	Polynuclear Neutrophiles	Polynuclear Neutrophiles, Degenerated	Monocytes	Transitional Cells	Basophiles	Eosinophiles	Small Lymphocytes	Large Lymphocytes
13	8/6-35	* N	-	7200	49	-	4	1	1	1	31	13
"	8/7-35	O	-	-	-	-	-	-	-	-	-	-
"	8/8-35	PO	.1 cc.P-3	31000	1	23	5	1	-	-	67	3
"	8/8-35	"	-	10800	-	-	-	-	-	-	-	-
"	8/9-35	"	.1 cc.P-3	13830	41	8	6	1	2	-	39	3
"	8/12-35	"	"	30950	28	1	1	2	1	-	66	1
"	8/13-35	"	"	8900	52	-	2	1	2	-	36	7
"	8/14-35	"	"	8000	48	-	3	-	-	-	37	12
"	8/15-35	"	"	9500	50	-	3	2	1	-	35	10
"	8/16-35	"	-	8500	53	-	-	-	-	1	42	4
"	8/19-35	"	-	9000	49	-	3	1	1	-	35	11
		D										
14	8/26-35	N	-	7500	43	-	5	1	1	-	40	10
"	8/26-35 p m	O	-	-	-	-	-	-	-	-	-	-
"	8/27-35	PO	-	13400	43	5	1	-	-	1	48	2
"	8/28-35	"	.5 cc.P-3	3600	4	8	2	-	3	-	71	12
"	8/29-35	"	"	17550	42	-	2	-	4	-	42	10
"	8/30-35	"	"	7700	54	-	5	3	5	-	28	5
"	9/3-35	"	"	8000	53	-	3	1	1	-	34	8
		D										
15	10/2-35	N	-	9400	52	-	2	1	-	1	37	7
"	10/2-35 p.m.	O	-	-	-	-	-	-	-	-	-	-
"	10/3-35	PO	-	6800	12	59	5	1	-	-	16	7
"	10/4-35	"	.1 cc.P-3	4000	-	25	3	-	-	-	67	5
"	10/5-35	"	"	9350	48	24	-	-	-	1	22	5
"	10/6-35	"	"	10750	54	4	7	2	2	-	25	6
"	10/7-35	"	"	9000	51	-	5	3	1	1	30	9
"	10/8-35	"	-	7500	48	-	3	1	-	-	38	10
"	10/9-35	D										

*Note:- N = Normal. O = Operated. PO = Post Operative. D = Discontinued.

RABBITS 16-17 --- Females, each weighing 3 Kgs. were used. These animals were prepared as the preceding ones, except that 10 cc. of a 24-hour culture of Streptococcus pyogenes grown in serum broth was placed into the Naturalaub skin sacs. Rabbit 16 developed the granulocytopenic condition, and after administering .1 cc. of P-3 intramuscularly for 4 consecutive days, recovered. Rabbit 17 did not develop a typical granulocytopenic condition. The second day after the operation the total leukocyte count was 21900, and only a small number of normal polynuclear neutrophiles could be found. After giving the rabbit .1cc. of P-3 intramuscularly daily for 4 days, the blood picture returned to normal and the animal appeared to be in good condition.

The data are given in Table 6.

TABLE 6

RESULTS ON P-3

Rabbits	Dates	Condition	Substance Administered	Total Leukocytes	Polymuclear Neutrophiles	Polymuclear Neutrophiles, Degenerated	Monocytes	Transitional Cells	Basophiles	Eosinophiles	Small Lymphocytes	Large Lymphocytes
16	10/2-35	* N	-	6800	48	-	4	1	1	1	33	12
"	10/2-35	O	-	-	-	-	-	-	-	-	-	-
"	10/3-35	PO	-	6500	7	58	-	-	3	-	32	-
"	10/3-35	"	-	6150	1	23	4	1	1	-	66	4
"	10/4-35	"	.1 cc.P-3	3900	4	12	-	-	-	-	80	4
"	10/4-35 p.m.	"	"	6800	37	2	3	3	1	2	48	4
"	10/5-35	""	"	6350	52	-	3	1	3	1	35	5
"	10/6-35	"	"	7200	43	-	-	-	2	-	47	8
"	10/7-35	"	-	8500	50	-	1	1	1	1	37	9
"	10/8-35	D										
17	10/2-35	N	-	8900	53	-	2	3	-	1	34	7
"	10/2-35	O	-	-	-	-	-	-	-	-	-	-
"	10/3-35	PO	-	7700	21	12	2	-	1	-	60	14
"	10/4-35	"	-	21900	6	29	3	-	1	-	57	4
"	10/4-35	"	.1 cc.P-3	14250	4	6	-	-	3	-	80	7
"	10/5-35	"	"	9500	15	5	2	2	1	1	70	4
"	10/6-35	"	"	8150	20	5	4	1	-	-	64	6
"	10/7-35	"	"	8000	48	1	3	-	2	-	36	10
"	10/8-35	"	-	7200	46	-	2	4	-	-	43	5
"	10/9-35	"	-	9100	49	-	-	1	2	1	36	11

*Note:- N = Normal. O = Operated. PO = Post Operative. D = Discontinued.

DISCUSSION

From these studies, it is evident that by cold saponification it is possible to isolate from yellow bone marrow a nonsaponifiable fraction which appears to possess granulocytopoietic properties. The foregoing data also show that this fraction is active when administered either orally or intramuscularly to animals in which a condition of granulocytopenia was produced experimentally. The data in Table 1 show that if the saponification is carried out at 80° to 100° C. the fraction has no activity. In addition, the data in Table 2 show that granulocytopoietic activity could not be demonstrated on whole yellow bone marrow, which had been melted at 40° C. This may be due to the fact that the maximum amount of marrow that could be administered to rabbits was insufficient to produce a stimulating effect upon the granulocytopoietic system. The fat solvents employed in Extractions 1 to 10 did not afford a means of separation, because as stated each solvent dissolved too much of the fat, thus preventing a concentration. Although, a very highly active concentrate was prepared, it will be necessary to conduct further studies on the purification and concentration, before any suggestion of chemical composition can be made.

In this work, attempts to produce granulocytopenic conditions in rabbits and rats by the use of benzene, amidopyrine, and hydroquinone were unsuccessful. Consequently, the method employed by Dennis (18) was used and proved to be satisfactory. Although, the condition obtained by this procedure may not be identical with that in humans, it affords a convenient laboratory method of demonstrating the granulocytopoietic activity of yellow bone marrow concentrations.

CONCLUSION

A highly concentrated fraction, possessing granulocytopoietic activity, was prepared from yellow bone marrow.

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