which would reduce the effective concentration of alloxan at any time.

Our results confirmed completely those of Kuhn and Quadbeck with regard to the effect of a first injection of alloxan with boric acid. We are unable to account satisfactorily for the discrepancy between our results and theirs on second injection. They found the second injection much more toxic than the first while we observed this in only a few cases. No effect of alloxan could be seen from most of our blood sugar values, but the mild hemolysis showed that it was not always completely inactivated. A somewhat more serious damage to the pancreas might have occurred in the animals of Kuhn and Quadbeck, not sufficient to cause frank diabetes but leaving them more susceptible to injury by the second dose. This is more probable since their dose of alloxan varied from 150 to 250 mg/kg while ours was always 160 mg/kg.

Summary. When rats on special rations were injected simultaneously with alloxan and boric acid, the deleterious effects of the alloxan were reduced. Only a few animals showed mild diabetes while alloxan alone produced severe diabetes in all cases. Survival for 7 days in animals receiving a high-lard diet was raised from 10% to 80%. Intravascular hemolysis in tocopherol-deficient animals on the high-lard diet, always severe with alloxan alone, was absent or mild when the boric acid was given.

17056

Transfusion of Leukocytes and Products of Disintegrated Leukocytes.*

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It is a common clinical observation that no demonstrable rise in leukocyte count can be obtained despite repeated transfusions of whole blood, even when given to individuals with marked leukopenia. It has been thought that this failure to successfully increase the leukocyte count by transfusion might be due either to the short life span of leukocytes, to their rapid disappearance from stored blood or possibly to type incompatibilities. This investigation was undertaken to determine whether transfused leukocytes would remain in the blood stream, and if not, to study the mechanism of their removal.

Methods. White blood cells were obtained from the rabbit's peritoneal cavity by a modification of the method of Mudd and coworkers.[1] 300 to 500 cc of physiologic saline were injected into the peritoneal cavity in the evening and a similar amount of saline the next morning about 15 hours later. Overdistension of the rabbit's peritoneum is poorly tolerated and may result in death of the animal. Four hours after the second injection of saline, the fluid was removed from the peritoneal cavity through a 16 gauge needle, using 0.5 cc (5 mg) of heparin as an anticoagulant. The fluid obtained was centrifuged at a low speed for 5 minutes and the sediment resuspended in 10 to 20 cc of Tyrode's solution so that the leukocyte count was in the neighborhood of 50,000 cells per cmm. This cell suspension was then administered intravenously to the same rabbit (autotransfusion) or into other rabbits (heterotransfusion). The cell free peritoneal fluid was also administered intravenously. The cells obtained by this method exhibited apparently normal ameboid movement, were actively phagocytic for *staphylococcus albus*, and took up vital stains. About 90 per cent

* This work was supported in part by a grant from the National Vitamin Foundation under the direction of Dr. A. D. Welch.

1 Mudd, S., Lucké, B., McCutcheon, M., and Strumia, M., *J. Exp. Med.*, 1929, **49**, 779.

TABLE I.
Autotransfusion: Transfusion of White Cells from Rabbit's Peritoneal Cavity into Ear Vein of Same Rabbit.

Rabbit No.	Control WBC ×100	Max. leukopenia ×100	Time onset leukopenia (min.)	Duration leukopenia (hr)	% leukopenia	Max leukocytosis ×100	Time onset leukocytosis (hr)	% leukocytosis
12	45	15	1	2	67	123	6	173
20	191	51	5	1	73	321	3	68
RP 1	59	12	1	4	65	(52)*	—	—
21	223	47	10	1	88	530	3	133
25	128	31	1	5	76	(100)*	—	—
26	104	44	5	5	58	(71)*	—	—
569	51	—	—	—	—	192	1	276
29	211	26	1	5	89	(82)*	—	—
31	70	18	1	½	73	148	4	116
33	134	39	1	3	70	(198)*	—	—
41	51	16	1	1	69	(50)*	—	—
43	32	7	1	1	78	81	3	149
46	30	19	1	1	38	85	6	179
48	58	32	10	1	45	(87)*	—	—
53	36	—	—	—	—	135	7	305
55	43	7	1	½	84	79	6	84
Avg	91.75	26.32	2.8	2.2	69.3	188.33†	4.3†	164.8†

* The figures in parenthesis are those which were not considered to represent a significant leukocytosis.
† Includes only those with a significant leukocytosis.

TABLE II.
Heterotransfusion: Transfusion of White Cells from Rabbit's Peritoneal Cavity into Ear Vein of Another Rabbit.

Rabbit No.	Control WBC ×100	Max. leukopenia ×100	Time onset leukopenia (min.)	Duration leukopenia (hr)	% leukopenia	Max leukocytosis ×100	Time onset leukocytosis (hr)	% leukocytosis
30	166	55	5	½	68	326	4	96
32	190	30	5	6	79	(148)*	—	—
34	67	37	5	2	43	151	3	122
40	235	54	1	2	79	748	3	218
44	268	65	1	4	75	(282)*	—	—
45	95	29	1	3	69	263	5	175
47	74	18	5	2	75	129	7	73
49	346	28	1	1	91	1262	3	235
54	158	20	1	2	87	(184)*	—	—
56	80	21	1	2	73	(88)*	—	—
57	108	28	1	4	74	(102)*	—	—
RP 9	118	51	1	2	57	194	3	64
RP 11	108	34	1	1	68	299	2	177
RP 13	119	44	1	1	62	(152)*	—	—
RP 15	81	7	1	2	79	(111)*	—	—
RP 16	93	19	1	4	74	(128)*	—	—
RP 17	103	32	5	2	69	248	3	208
Avg	144.17	33.70	2.2	2.2	72	403.50†	3.7†	152†

* The figures in parentheses are those which were not considered to represent a significant leukocytosis.
† Includes only those with a significant leukocytosis.

of the cells were mature neutrophils.

Disintegrated leukocytes were obtained by subjecting the resuspended leukocytes to supersonic vibration for 1 hour at 0°C. The material obtained was centrifuged and the supernatant fluid, free of particulate matter, was administered intravenously to rabbits.

Results. Transfusion of leukocytes into the ear vein of the same rabbit from which they had been obtained resulted in a sudden profound leukopenia in 14 of 16 rabbits (Table I). A transient unsustained drop in

TABLE III.
Transfusion of Disintegrated White Cells.

Rabbit No.	Control WBC ×100	Max. leukopenia ×100	Time onset leukopenia (min.)	Duration leukopenia (hr)	% leukopenia	Max. leukocytosis ×100	Time onset leukocytosis (hr)	% leukocytosis
6	168	43	1	3	74	333	4	98
7	225	64	1	½	71	448	1	98
12	172	45	1	5	79	(142)*	—	—
14A	152	24	1	6	81	(176)*	—	—
14B	175	30	1	6	88	(174)*	—	—
15A	34	11	1	2	69	143	3	379
15B	183	84	1	½	97	476	1	105
16	78	16	1	5	79	(74)*	—	—
17	267	27	1	1	90	389	2	49
21	173	33	1	½	80	286	1	65
Avg	163.05	37.70	1	2.95	80.8	345.9†	2†	132†

* The figures in parentheses are those which were not considered to represent a significant leukocytosis.

† Includes only those with a significant leukocytosis.

TABLE IV.
Summary of Data.

Type of transfusion	Avg control WBC	% developing leukopenia	Avg leukopenia	Avg % drop in WBC	Avg leukocytosis	% developing leukocytosis	Avg % increase in WBC
Autotransfusion	9,175	88	2,632	69.3	18,833	56	164
Heterotransfusion	14,417	100	3,370	71	40,350	53	152
Disintegrated WBC's	16,305	100	3,770	80.8	34,590	60	132

the leukocyte count occurred in the other 2 animals. An initial elevation of the leukocyte count did not occur in any animal. The average time of onset of the leukopenia was 2.8 minutes, the average duration 2.2 hours and the average drop in leukocyte count 69.3% of the control count. In 9 of the 16 rabbits (56%) a subsequent leukocytosis occurred. In these rabbits the average rise in leukocytes was 164.8% of the original leukocyte count and the average time of onset was 4.3 hours after the transfusion was given.

When the cell suspension was transfused into the ear vein of another rabbit, a marked leukopenia developed rapidly in all instances (Table II). The average drop in white count was 72% of the control count, the average time of onset 2.2 minutes, and the average duration 2.2 hours. In 9 of 17 rabbits a subsequent significant rise in leukocyte count occurred. The average maximum rise in leukocyte count was 152% of the initial value and the average time of onset was 3.7 hours.

Intravenous administration of the supernatant fluid obtained by centrifugation of disintegrated leukocytes also produced a leukopenia in all instances (Table III). The average drop in white count was 80.8% of the control count, the average time of onset was 1 minute, and the average duration was 2.95 hours. A subsequent leukocytosis occurred in 6 of 10 rabbits. The average maximum rise in white count was 132% of the control count and the average time of onset of the rise in white count was 2.0 hours. The results of autotransfusion, heterotransfusion and transfusion of disintegrated white cells are summarized in Table IV.

The drop in white count was accompanied by a decrease of approximately 20% in neutrophils and a corresponding increase in the percentage of lymphocytes in the differential count. During the phase of leukocytosis there was a marked increase in the number of neutrophils (to 90% or more) with a shift to the left.

Administration of normal saline, Tyrode's solution, whole blood, heparin, killed typhoid bacilli and a solution of desoxyribosenucleic acid intravenously did not proluce any sig-

nificant change in the leukocyte count. 10 to 15.0 cc of a 1.1% solution of sodium citrate always produced a marked leukopenia but was never followed by leukocytosis.

Mixture of whole blood and white blood cell suspension *in vitro* resulted in the calculated rise in the total white count with no significant change in the count over a period of several hours. It was not possible to demonstrate any differences in white blood cell types by cross-matching with the sera from various recipient rabbits.

Transfusions with intact leukocytes as well as with products of leukocytic disintegration frequently resulted in sudden death, preceded by nystagmus and convulsions in all cases. Rabbits used for repeated transfusions lost weight, appeared chronically ill, and frequently died. The cellular debris from disintegrated white cells appeared to be the most toxic of the substances used. When cell suspensions contained a high percentage of cells showing toxic granulation and disintegrating cells as determined by examination of smears, toxic reactions appeared to be more common than if such were not the case. The thromboplastic activity of leukocytes disintegrated by supersonic vibration was determined. The greatest thromboplastic activity was found in the centrifuged sediment. 0.1 cc of the material was equivalent to 0.940 mg of Maltine thromboplastin; in contrast, 0.3 cc of the supernatant material from disintegrated leukocytes contained less than 0.1 mg. In spite of the high content of thromboplastin, intravenous clotting was not observed in any animal.

Because of the histamine content of leukocytes[2] the effect of intravenous histamine phosphate on the white count was investigated. 0.2 to 0.6 mg of histamine phosphate produced a mild delayed leukopenia in 3 of 10 rabbits. Neither the time of onset, the degree of leukopenia nor the duration of the leukopenia corresponded to that produced with the transfusion of intact leukocytes or the products of their disintegration. One mg of histamine intravenously resulted in sudden death with nystagmus and convulsions. The intravenous administration of an anti-histaminic (pyranisamine maleate) did not prevent the characteristic leukopenia which developed with the transfusion of a white cell suspension in 2 rabbits.

Discussion. It is evident that there is a potent substance (or substances) present in leukocytes obtained from the rabbit's peritoneal cavity which is capable of affecting the level of the leukocyte count of circulating blood. Not only do the transfused cells disappear from the circulation but white cells circulating prior to the transfusion disappear as well. This, together with the results obtained with particle-free solutions of lysed cells would indicate that the effect is more than a mere filtering of foreign bodies by the reticuloendothelial system.

The fate of the transfused cells has been studied in preliminary experiments using leukocytes labeled with radioactive phosphorus. With this method the greatest concentration of radioactivity is found in the lungs. This finding is confirmed by histologic studies of lungs removed at the time of maximum leukopenia. The lung capillaries were found to contain large numbers of leukocytes without edema or fibrin formation. The liver and spleen also contained increased numbers of leukocytes but this finding was not as marked as in the lungs.

Menkin[3,4] has described a leukopenic and leukocytic factor, as well as a toxic substance called necrosin, obtainable from inflammatory exudates. It is possible that these substances obtained from exudates are derived entirely from disintegrating leukocytes. It is noteworthy that effects obtained by administration of leukocytes produced with physiologic salt solution are similar to those obtained by exudates produced with irritants.

It cannot be stated whether leukocytosis is a response to a specific agent contained in the transfused leukocytes or their products, whether it is a response on the part of the bone marrow initiated by the leukopenia or whether it is a spontaneous variation. The fact that leukocytosis does not develop in all

[2] Code, C. F., *J. Physiol.*, 1937, **90**, 485.

[3] Menkin, V., *Arch. Path.*, 1946, **42**, 154.

[4] Menkin, V., *Science*, 1947, **105**, 538.

animals in which leukopenia has been produced by administration of leukocytes or their products, and in no animal in which leukopenia was induced with sodium citrate solution, argues against the subsequent leukocytosis being a response of the bone marrow stimulated in some manner by the leukopenia. The possibility of the existence of differences in the reaction of the recipient is indicated by the production of leukocytosis in one rabbit but not in another with the same cell suspension.

The chemical nature of the active substances present in solution of lysed leukocytes has not as yet been determined. Preliminary experiments indicate that removal of lipids, including thromboplastin, by ether extraction does not reduce its activity. Considerable loss of activity results from exposure to heating at 66°C for 1 hour.

Summary and conclusions. 1. Leukocytes were obtained in large numbers by introduction of large amounts of physiologic salt solution into the peritoneal cavity of rabbits.

2. Intravenous administration of these cells to the same or different animals was followed by very rapidly developing and severe leukopenia. A subsequent leukocytosis developed in the majority of rabbits after several hours.

3. Disintegrated leukocytes, or aqueous extract of disintegrated leukocytes produced similar results.

4. Preliminary data indicate that the leukocytes stick in the capillaries of the lung. Ether extraction of disintegrated leukocytes does not cause loss of activity but heating reduces activity.

5. These experiments indicate that unsatisfactory preservation of leukocytes in stored blood is not the reason for failure to raise leukocyte counts with transfusions, but that white blood cells contain a substance (or substances) which is capable of producing leukopenia.

We are indebted to Dr. William Holden for the assay of the thromboplastic content of leukocytes.

17057

Effect of Adrenocorticotrophic Hormone Upon Liver Fat and Urinary Phosphorus in Normal Force-Fed Rat.

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It was shown by Baker, Ingle, Li, and Evans[1] that the administration of pure adrenocorticotrophic hormone (ACTH) to force-fed male rats caused fatty infiltration of the liver as was demonstrated histologically by Sudan stains. This observation was confirmed by chemical analysis of the liver in the present study. In addition, during the administration of ACTH there was noted a significant increase in the urinary excretion of inorganic phosphorus which accompanied a rise in urinary nitrogen and that there was excretion of glucose and loss in body weight. Changes in the weights of certain organs was noted.

Methods. Male rats of the Sprague-Dawley strain were maintained on a diet of Archer Dog Pellets until they reached a weight of approximately 300 g. They were then placed in metabolism cages and maintained on a fluid diet administered by stomach tube each morning (8:30 to 9:15 A.M.) and afternoon (4:15 to 5:00 P.M.). The technic of force-feeding and the diets used were modi-

[1] Baker, B. L., Ingle, D. J., Li, C. H., and Evans, H. M., *Am. J. Anat.*, 1948, **82**, 75.