A STUDY OF THE CHANGES IN THE BLOOD AND
BLOOD-FORMING ORGANS PRODUCED BY CYTO-
TOXIC SERA, WITH SPECIAL REFERENCE TO
HÆMOLYMPHOTOXIN.¹

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PLATES IV AND V.

Since this article was originally planned, many changes have been made in its scope and general outline, not only because the great amount of study devoted to the cytotoxins has rendered some of the work first planned unnecessary, but also because the opinions concerning the cytotoxins, their mode of action, specificity, etc., have undergone many changes. Perhaps the most radical change of ideas is in regard to specificity, many observers having bent their energies towards definitely proving or disproving the specificity of the cytotoxins. The one who has perhaps done most towards proving the non-specificity of the cytotoxins is Pearce, who, in a recent paper before the American Association of Pathologists and Bacteriologists, has clearly demonstrated that the changes which have been regarded as specific by most writers are due to hæmoly-sins and agglutinins; when these factors, the former in particular, are considered, the changes cannot be regarded as specific,—they are not changes in kind, but are rather changes in degree alone.

In the investigations here reported, an attempt is made to compare several cytotoxins to find what changes they produce, and, if the changes are merely in degree, to determine to what extent these changes occur after the injection of different cytotoxins, and the order in which the degree of the change would

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place them. Furthermore, an attempt is here made to produce a hæmolymphocytotoxin and by its aid to throw if possible some light upon the physiological action of the hæmolymph glands. So far no attempt has been made to produce a hæmolymphocytotoxin and to study the effects produced by it; in doing so an entirely new field is entered.

Taken for granted that the hæmolymph nodes are organs *sui generis*, as demonstrated by Warthin (1), what then is their true function? Some of the theories may be stated as follows:

1. They are hæmolytic, and therefore destructive organs, and rid the body of old and partially broken-down red blood cells.
2. They are centres for the formation of leucocytes and blood plasma, and in these respects conservative.
3. They are centres for the production of large numbers of eosinophile cells; the granules arising from the disintegration products of red cells are taken up by mononuclear leucocytes, the nuclei of which become polymorphous.
4. They compensate in some degree for the spleen in case of inability of that organ to perform its proper function.
5. They are centres for the destruction of leucocytes.
6. Since they are enlarged in certain infections, they play a part in the production of immunity and in the protection of the body through the formation of anti-bodies.

The suggestion by Warthin and Lewis (2) of a hæmolytic ferment produced by these glands brings forth more strongly the possibility that they play an important part in the production of certain forms of anæmia. In apparent confirmation of this suggestion, Warthin, in eight autopsies on cases of pernicious anæmia, has found the hæmolymph nodes exceedingly red and hyperplastic and showing signs of over-activity. One object of this paper, then, is to prove if possible the presence of such a ferment; for, if it is present, a more hæmolytic serum should be produced by the injection of hæmolymph nodes than would be produced by injection of defibrinated blood, diluted to a corresponding percentage of red blood cells.

Throughout this work it will be frequently necessary to refer to the effects of lymphotoxin and myelotoxin on the blood and

blood-forming organs. The literature of these subjects has of late become so extensive that a review of it here is impossible; some of its aspects, however, have been most excellently reviewed in the articles by Flexner (3) and by Bunting (4) to whose work references will often be made.

METHODS AND MATERIALS.

The basis of the present investigation is that a specific toxin for a tissue may be formed by the injection of that tissue into an intermediary animal. Glands were obtained from sheep and injected into geese; the serum of the goose, after a sufficient time had elapsed for attaining its highest toxicity, was injected into a sheep, the tissues of which were then examined for evidences of a specific toxin.

In producing the toxin geese and sheep were used, the former on account of their ability to withstand repeated injections without becoming infected, and the latter because the hæmolymp glands obtained from them are larger and more numerous than from the animals ordinarily used for experimental purposes.

The geese received suspensions of glands prepared by the method in general use. Glands removed under as nearly sterile precautions as possible were first triturated in a sterile mortar, suspended in sterile physiological salt solution, and allowed to extract for twelve hours; they were strained through sterile gauze and injected into geese. The injections were made intraperitoneally at intervals of from three to ten days until seven or eight injections had been made; at the end of this time the geese were bled.

When the geese were injected with blood, blood was first defibrinated and then diluted with physiological salt solution in such quantity that the percentage of red blood cells was the same as that contained in a corresponding injection of hæmolymp nodes, so that the results might be comparable.

The geese used were as follows:
Goose 2 furnished normal serum.
Goose 5 furnished normal serum.

Goose 3, 4, 6, and 12 each received seven injections of hæmolymp glands, extending over a period of about thirty-six days.

Goose 7 and 14 received seven injections of defibrinated blood diluted as above.

Goose 11 received 6 injections of lymph glands.

Goose 15 received 8 injections of endothelium.

Goose 16 received 5 injections of liver.

Goose 17 received 5 injections of kidney.

Goose 18 received 5 injections of spleen.

(Note.—Goose 1 and Sheep 1 were used in a preliminary experiment performed a year ago, but not included in the present series of experiments.)

NORMAL HISTOLOGY OF THE SHEEP.

The tissues to be examined were removed from normal sheep, fixed in formalin and mercuric chloride, and stained with hæmatoxylin and eosin. These methods were uniformly applied to the pathological organs. Various special stains were also employed when deemed necessary. For the iron reaction, potassium ferrocyanide and hydrochloric acid were used.

Blood.—Counts of the total number of leucocytes as well as differential counts were made shortly before injection of the sheep and at varying intervals for four or five days after. At the end of this time the sheep were killed and sections made from all the organs. In counting the cells of the blood, the Thoma-Zeiss apparatus was used and at the same time smears were made for differential counts, the latter being fixed in alcohol and ether and stained with methylene blue and eosin.

The average leucocyte count of a sheep is about 8000, but varies between the wide limits of from 6000 to 12,000 per cubic millimetre. The erythrocyte count may also vary greatly and still be within normal limits, but averages eight million per cubic millimetre.

In order that the results might be comparable, the following cells were taken into consideration in making the differential counts, this classification of leucocytes being used by Bunting in his work on the blood of the rabbit:

- 1.—Mononuclear Leucocytes,
 - a—Lymphocytes,
 - b—Large Mononuclears,

- 2.—Polymorphonuclear Leucocytes,
 a—Pseudo-eosinophiles (Amphophiles),
 b—Eosinophiles (coarsely granular),
 c—Basophiles (Mast Cells).

The last are so few in numbers in the sheep that they might well be left entirely out of consideration. The average percentage and limits of variation as obtained from about twenty counts from normal sheep may be given as follows:

Lymphocytes.....	53	%	40	to	60	%
Large Mononuclears....	8	%	3	to	11	%
Amphophiles.....	37	%	30	to	55	%
Eosinophiles.....	1.7	%	.2	to	8	%
Mast Cells.....	.3	%	0	to	2	%

It is readily seen from the above that the different forms may vary within rather wide limits and still be normal.

Hæmolymph Glands.—The hæmolymph glands of the sheep are typical in structure, the majority being of the splenolymph type. They have a thick capsule from which trabeculæ, forming either a delicate framework or a coarse framework densely infiltrated with lymphoid tissue, extend into the gland, dividing it into irregular lobules. The lymphadenoid tissue, while varying greatly in amount, is usually abundant and forms the greater part of the gland. As a rule, large numbers of nodes with germ centres showing fairly active proliferation are present, although in a few cases cords alone are found. The peripheral blood sinus is generally large but may be frequently interrupted by lymphadenoid tissue projecting into it. The gland shows a few phagocytes, some pyknotic and vesicular nuclei, and a light sprinkling of eosinophiles, the latter being for the most part polymorphonuclear, although a few mononuclear forms are to be found.

Lymph Glands.—The lymph glands are almost identical with human glands in structure, having a thick cortical substance composed of lymph nodules and germ centres, and a medullary substance composed of anastomosing cords separated by trabeculæ. The entire gland is surrounded by a rather thick capsule.

Only a few eosinophile cells are seen and these are chiefly around the borders of the lymph nodes.

Spleen.—The spleen has a thick capsule, and the trabeculae throughout the organ are conspicuous. The Malpighian bodies are large, distinct, and not very numerous. The lymphoid cells in the pulp are scanty, the reticulum being relatively increased, and the reticular cells large; the organ seems to show an overabundance of connective tissue.

Bone Marrow.—The bone marrow cells found in the sheep having practically the same characteristics as those of the rabbit, the following classification as given by Muir (5) for the latter animal may here be used:

1.—Leucocytic class of cells:

a—Cells with finely granular protoplasm which constitute the great majority of the cells in the marrow. After mitotic division they become the finely granular amphophile leucocytes of the blood.

b—Cells with coarse eosinophile granules which later become the ordinary eosinophiles of the blood.

c—Hyaline or non-granular cells.

Small lymphoid cells.

Large lymphoid cells.

Large mononuclear cells.

2.—Erythrocytes.

3.—Erythroblasts:

a—Megaloblasts or large nucleated red corpuscles faintly tinted with hæmoglobin; these are the youngest variety of erythroblasts.

b—Normoblasts or typical nucleated red corpuscles, which are somewhat larger than red blood cells and are the oldest variety of erythroblasts.

4.—Giant Cells.

5.—Connective-tissue class of cells, fat cells, etc.

EXPERIMENT WITH PHYSIOLOGICAL SALT SOLUTION.

As noted by Besredka (6), Ricketts (7), Brinckerhoff and Tyzzer (8), and later by Bunting, injections of fluids intra-

peritoneally cause first a leucopenia and later a hyperleucocytosis of the peritoneal fluid. It is probable that within certain limits the greater the toxicity of the fluid injected the greater the resulting hyperleucocytosis. (Ricketts.) The bone marrow and lymph glands in attempting to compensate for this withdrawal of leucocytes from the circulation give rise to a rather typical blood picture. Bunting found after the injection of cold sterile physiological salt solution a slight rise in the total leucocyte count of the circulating blood. On differential analysis this increase was found to be due to a marked amphophile leucocytosis, at the same time that there was a correspondingly great lymphopenia. The preliminary leucocytosis he calls the "anticipatory" leucocytosis, and is due to the reaction of the bone marrow to the withdrawal of leucocytes from the circulation, the lymphocytes having a slower amoeboid movement failing to respond so quickly.

As these experiments were performed on rabbits it was thought best to repeat them with sheep so that the influence of salt solution might be exactly determined for that animal and could then be eliminated from the final results. After the injection of thirty-five cubic centimetres of cold sterile physiological salt solution (Table I),* a rather sharp leucocytosis and a return to normal within twenty-four hours is obtained, and is followed by another lesser rise and fall in the next forty-eight hours. By differential counts we find at first a typical "anticipatory" leucocytosis with its accompanying lymphopenia, the lymphocytes, however, recovering quite rapidly. The second leucocytosis is due to an increase both of the amphophiles and of the lymphocytes with a final rather gradual return of both to normal.

The bone marrow, then, in the sheep as well as in the rabbit, reacts quickly to a demand upon it, the lymphocytes, because of their slower amoeboid movement, responding more slowly.

INJECTION OF NORMAL SERUM OF THE GOOSE.

In the following experiments no consideration has been taken of the amount of hæmolysis *in vitro*, this being reserved for a

* The tables are given at the end of the article.

future study. The blood curve (Table II) resulting from the injection of normal serum is almost identical with that obtained by Bunting, showing a marked leucocytosis at the end of eighteen hours, followed by a fall, preceding a second and finally a third slighter rise. On differential analysis the first rise is found to be due almost entirely to an amphophile leucocytosis, except for a sharp rise of lymphocytes, with a sharper return to normal, occurring within eight hours. This preliminary increase in lymphocytes is in marked contrast, however, to the observations of Bunting, who found a typical "anticipatory" leucocytosis, as described, with its accompanying lymphopenia. The second and third rises are due entirely to an increase in lymphocytes; the number of amphophiles decrease and subsequently there is only a slight secondary rise to normal.

The large mononuclear leucocytes increase in numbers following roughly the rise and fall of the lymphocytes; the eosinophiles, after a preliminary sharp decrease in numbers, gradually rise considerably above normal and then decrease; the erythrocytes show a slight increase at first and subsequently their number remains elevated; the basophiles are so few in the sheep that they have not been enumerated in the table.

SHEEP 2.—Received 35 cc. of serum from Goose 5 at 3 P.M., December 1, 1903. A record of the leucocyte counts and differential analysis is given in Table II. The animal was killed December 8, 10 A.M. *Autopsy.*—There is slight oedema near the site of inoculation. A large amount of fluid is present in the peritoneal cavity. The hæmolymp glands are exceedingly large and numerous. All the organs and tissue are apparently normal. There is no infection.

Histology.—The structures examined showed little or no changes other than a slight hyperplasia. This is perhaps most noticeable in the bone marrow, as here the actual amount of cellular marrow is increased, the increase corresponding to a diminution in the number of fat cells. The change is due to an increase in number of the marrow cells or myelocytes. The megaloblasts and normoblasts are also more numerous than usual and show mitotic figures. The germ centres in the spleen and lymph glands stand out distinctly and show active although not excessive proliferation. Few, if any, degenerative changes are present.

The injection of normal serum of a goose into a sheep causes first a diminution in the number of the leucocytes in the circulating blood as a result of their withdrawal into the peritoneal

cavity. This fall is followed by a marked rise, due to proliferation of the antecedents of the leucocytes, first and most markedly in the bone marrow and later in the lymph glands, spleen, and hæmolymp glands. At the same time there is a slight increase in number of erythrocytes in the blood due to a proliferation of the erythroblasts in the bone marrow. The changes produced are chiefly hyperplastic, as few, if any, degenerative changes are apparent.

EXPERIMENTS WITH HÆMOLYTIC SERUM.

Hæmolytic serum was prepared by injecting a goose with normal defibrinated sheep's blood diluted with normal salt solution until it contained the same percentage of erythrocytes that is present in a corresponding injection of hæmolymp nodes.

The leucocyte curve (Table III) in this case is not very characteristic, consisting of a preliminary leucocytosis of about 2000 per cubic millimetre, followed by a still smaller increase and then a gradual rise covering a period of about seventy hours. By differential counts we find a typical "anticipatory" leucocytosis with its accompanying lymphopenia, after which there is a decrease in amphophiles and an increase in lymphocytes; the last two leucocytoses are due to the increase of lymphocytes.

The eosinophiles, after a preliminary decrease in number, gradually increase considerably above normal and then, as if a crisis had been reached, suddenly return to normal, at the end of about ninety-six hours.

The erythrocytes, after a rapid preliminary rise of one million, drop in twelve hours about two million and gradually decrease in the next thirty-two hours a million more, making a total destruction of about three million erythrocytes per cubic millimetre, or 28 + % of the entire number of corpuscles.

SHEEP 9.—Received intraperitoneally 35 cc. of serum from Goose 7 at 11.30 A.M., February 9, 1904. At 3 P.M. the sheep appeared sick. The blood is dark in color; at 10.30 P.M. the blood is light in color and watery. February 11, at 5 P.M., one normoblast is found in a differential count of 500 cells. February 12, 9 A.M., two normoblasts are seen. February 14, 9 A.M., one normoblast is found. The sheep was killed February 15, 11.30 A.M. *Autopsy.*—There is

no excess of peritoneal fluid. The hæmolymp glands are dark in color. The bone marrow is light in color. Axillary, mesenteric, and retroperitoneal lymph glands are dark in color. All other organs and tissues are apparently normal. There is no infection.

Histology.—The hæmolymp glands show few changes resulting from the injection of hæmolytic serum. The glands are somewhat congested and show evidences of increased erythrocyte destruction; large endothelial phagocytes are filled with pigment and partially broken-down corpuscles. Nuclear destruction in the lymphoid tissue is perhaps somewhat more abundant than normal, and large vesicular and in some cases pyknotic nuclei are present. The eosinophiles are increased in number.

The lymph glands show distinct evidences of proliferation in the germ centres, which are large and well developed. Evidences of erythrocyte destruction are much more marked than under normal conditions. Numerous large brown pigment granules are present, for the most part within phagocytes. Numerous vesicular and pyknotic nuclei and phagocytes containing broken nuclei are also present. There is some increase in eosinophiles.

The bone marrow shows an increase of mature amphophiles together with rather marked increase of the myelocytes. There is also a distinct increase of megaloblasts and normoblasts. In all of the tissues examined there are rather marked proliferative changes in the endothelium.

The injection of a hæmolytic serum into a sheep not only causes an increased destruction of erythrocytes, but has an effect on the leucocytes and indirectly at least on the leucocyte-forming organs. Although the changes in these organs are not pronounced, and in this case, at least, not very characteristic, they are nevertheless evident. The increase in both lymphatic and hæmolymp glands of endothelial phagocytes containing red cells and pigment is the most striking histological change found. In connection with these evidences of hæmolysis within cells is to be noted the increase of eosinophiles.

EXPERIMENTS WITH HÆMOLYMPHOCYTOTOXIC SERUM.

In two instances sheep were injected with hæmolympocyto-toxic serum. The first gave a more typical alteration of the leucocytes, but the animal was killed shortly after injection, in order to obtain tissues at an early stage; in the second animal the leucocytes were allowed to return to normal.

In these experiments changes undergone by the leucocytes (Tables IV and V) have resembled those which follow the injection of normal serum of the goose, but the increase which first

occurs takes place in a shorter length of time, as if the demand has been more urgent and the tendency to proliferate given a sharper impetus. After the first rise there is a complete return to normal, followed by a second slower and slighter leucocytosis, which in turn, after twenty-four hours, is followed by a third rise.

On differential counts a typical "anticipatory" leucocytosis with its accompanying lymphopenia is observed. The first rise already described is due entirely to an amphophile leucocytosis; the second, however, is due to a lymphocytosis, and the third to a combined increase of both amphophiles and lymphocytes. It is readily seen that, with the exception of an "anticipatory" leucocytosis, these changes are almost identical with those produced by the injection of normal serum of the goose, until the third rise is reached. In the case of the normal serum this is due entirely to a lymphocytosis, while with the hæmolymphotoxic serum it is due to a combined rise of lymphocytes and amphophiles.

The large mononuclears remain practically unchanged throughout the experiment. It is noteworthy that in every case cited the eosinophiles, after a preliminary decrease, gradually reach a number considerably above normal and then, as by a crisis occurring at the end of about ninety-six hours, suddenly return to normal.

There is a marked difference between the erythrocyte curves in the two experiments. In the first there is a slight rise occurring within the first four hours and then a decided drop. Great emphasis is to be laid on this marked decrease in number of erythrocytes, their number dropping from 9,215,000 to 4,824,000 in four hours—a fall of 47 + % of entire number of corpuscles. In the second curve, however, there is a preliminary increase followed by a slight decrease, another rise, and then a decided drop from 15,760,000 to 13,400,000 in twelve hours, there being altogether a destruction of 2,360,000 corpuscles, or about 15% of the entire number present. In both instances after this marked decrease in number there is a gradual rise to normal, and in both instances this regeneration is somewhat excessive, the erythrocyte count exceeding normal.

SHEEP 3.—Received 35 cc. serum from Goose 3 at 2 P.M., November 6, 1903. At the time of the count on November 6, at 10.30 P.M., the blood was noticeably lighter in color and more watery than normal. The animal was killed November 10, at 10 A.M. *Autopsy* showed slight œdema around the site of inoculation. A large amount of fluid was present in the peritoneal cavity. All of the lymph glands, with the exception of the hæmolymph glands, were large, hard, and extremely dark in color.

The spleen is small, being not over two-thirds its normal size. One kidney is markedly atrophic, the other showing compensatory hypertrophy. On section the pelvis is large and dilated; the cortex is thin. The liver is large, dark in color, and bleeds freely on section. The bone marrow is normal in appearance. There is no infection.

Histology.—The hæmolymph glands show a disappearance, or at least a diminution, in size and numbers of the distinct proliferating germ centres found in normal glands. In twenty-five glands examined exceedingly few germ centres are found, and those present are small and show few mitotic figures. The blood sinuses are somewhat congested and contain large numbers of leucocytes. Eosinophile cells (Fig. 2) are present in the lymphoid tissue in large numbers; most of them are polymorphonuclear, but some are mononuclear, and a few even polynuclear. There is less evidence of cell destruction in the hæmolymph glands than might be expected, only a few large vacuolated and some pyknotic nuclei being seen. Abundant endothelial proliferation is found, and large phagocytes containing broken-down erythrocytes and blood pigment are present in great numbers.

The germ centres in the lymph glands, while more numerous than in the hæmolymph glands, are small and do not show active proliferation. There is, however, an enormous amount of proliferation in the endothelium. The amount of blood pigment in some of the lymph glands is enormous, giving the glands on macroscopical examination a dark brown or blackish color. The pigment on microscopical examination (Fig. 3) is found in the medullary portion of the gland and is chiefly intracellular, having been taken up by phagocytes. It does not contain iron, and is probably hæmatoidin formed as the result of erythrocyte destruction. Other evidences of cell destruction are not more frequently observed than in the hæmolymph glands. There is considerable increase in the number of eosinophiles present.

In the spleen, as in the other tissues examined, the germ centres are small and are not actively proliferating. The spleen is very markedly atrophic, but this atrophy must have antedated the time of injection. Some pigment is present which, in sharp contrast to that found in the lymph glands, is iron-containing and chiefly extracellular.

The bone marrow shows a marked diminution in the number of mature cells, i. e., cells ready to enter the circulation. The marrow cells, with few exceptions, however, are in some stage of mitotic division. Megaloblasts and normoblasts are also increased in numbers, the former showing active proliferative changes. The endothelium is proliferating actively. Eosinophiles are decreased in numbers. A few large vacuolated nuclei are to be seen.

SHEEP 6.—Received 35 cc. serum from Goose 6 at 1 P.M., January 22, 1904. There was evidence of hæmoglobinuria at the end of forty-eight hours. The

animal was killed January 29, 10 A.M.; at autopsy the organs were apparently normal.

Histology.—Since the sheep was killed seven days after injection, the tissues may be expected to show either a greater attempt at repair or a later stage of the intoxication.

The hæmolymp glands in this experiment, as in the last, show a decided diminution in size and numbers of germ centres. Evidences of cell destruction here, however, are very striking. With very few exceptions every nucleus, though not necrotic, is in some stage of fragmentation. Pyknotic, large vesicular, and vacuolated nuclei are in great abundance. Numerous phagocytes containing particles of broken-up nuclei are present. The pigment, phagocytes, and eosinophiles have the same characters as in Sheep 3.

The lymph glands show the changes described for Sheep 3, except that there is more evidence of nuclear destruction; there are vacuolated, vesicular, and pyknotic nuclei, and endothelial phagocytes are found. Blood pigment is present in considerable amount, but not to the same extent as in Sheep 3, although the phagocytes are as numerous.

The spleen in this case shows more marked nuclear destruction than in Sheep 3. A much larger amount of pigment (Fig.4) is, however, present here; it is iron-containing, and is probably hæmosiderin.

The red bone marrow is not abundant. Proliferative changes are confined chiefly to the megaloblasts. The myelocytes are not numerous, and those present do not show very active mitosis. Fully developed amphophiles are, however, numerous. Eosinophile cells are decreased in number.

After the injection of hæmolympphotoxic serum there is, as with the injection of normal serum from the goose, a sharp demand on the leucocyte-forming organs, which is responded to first by the bone marrow and later by the lymphocyte-forming organs. The bone marrow is able, in some degree, to compensate for this loss by proliferation, but the lymphocytes in the blood seem to be solely supplied by preformed cells. Histological examination seems to show that the tendency to proliferation of the cells antecedent to the lymphocytes is inhibited. This inhibitory or toxic action, while slight at first, becomes exceedingly destructive, as shown by the marked signs of degeneration in the lymphadenoid tissue.

The differences in changes affecting the erythrocytes in the two experiments may be partly due to the pathological condition of the kidneys in Sheep 3. In both experiments there has been a preliminary rise in the number of erythrocytes, then a fall, and finally a slow recovery which becomes excessive and carries the erythrocyte curve above normal. In Sheep 3, the

kidneys being pathological, it is probable that the excretion of the toxin has not been carried on to the same extent as in Sheep 6. Since this animal has been able to excrete some part of the toxin, the effect has occurred slowly and is less marked.

Evidence of erythrocyte destruction in the tissues is somewhat more marked in Sheep 3 than in Sheep 6, but this difference again is partly referable to the kidneys of the former; for in the latter hæmoglobinuria was present, while in Sheep 3 there was no evidence of it. The regeneration of erythrocytes is explained by the marked proliferation of megaloblasts in the bone marrow.

PARALLEL EXPERIMENTS WITH HÆMOLYMPHOTOXIN AND WITH
HÆMOLYSIN.

Control experiments were here tried with hæmolympphotoxic and erythrolytic serums with the special purpose of determining the amount of hæmolysis in either case and of making careful comparisons.

To this end for corresponding experiments geese and sheep were selected respectively as nearly alike as possible; they had been kept under similar conditions. The geese were injected with the same amount of fluid in each case, and each injection contained the same amount of blood as determined by Gower's hæmoglobinometer; the goose injected with emulsion of hæmolymp gland received the same number of erythrocytes as the one receiving the blood. The conditions of the experiments were made parallel in every respect, so that the results obtained might be exactly compared.

I. *Experiment with Hæmolympphotoxin*.—Changes in the number of leucocytes (Table VI) obtained by injecting hæmolympphotoxin from the goose selected resemble in general those of Table IV, obtained after injection of hæmolympphotoxin from Goose 3; twice their number rises markedly. Examination of differential counts shows that the preliminary leucocytosis in both cases is due at first to an amphophile leucocytosis, later aided by a lymphocytosis. The second rise, however, is markedly different in the two cases, for in one instance it is

entirely due to an amphophile leucocytosis, while in the other it is wholly a lymphocytosis.

The erythrocytes in Sheep 12 after a preliminary rapid rise gradually but steadily decrease for fifty-six hours and then as gradually return to normal. Altogether there is a drop from 4,800,000 to 3,240,000, or a total destruction of 32% of the entire number of erythrocytes present.

The eosinophiles show the usual preliminary decrease, the gradual rise, and the so-called crisis.

SHEEP 12.—Received 40 cc. serum from Goose 12 at 1 P.M., April 23, 1904. Killed April 28, 4 P.M. *Autopsy.*—Hæmolymph glands are not numerous, but are large; lymph glands are rather dark in color. The liver has a peculiar greenish tinge. The bone marrow is anæmic. Otherwise the organs are normal and there is no infection.

Histology.—In sharp contrast to the other instance in which hæmolymphocytotoxin has been injected, this one shows the germ centres in the hæmolymph glands to be large, numerous, and actively proliferating. In other respects, however, they resemble those already described, in that there are a large number of degenerative changes, marked proliferation of endothelium, many phagocytes containing blood pigment and corpuscles, and a marked increase of eosinophiles.

In the lymph glands again the germ centres are large, numerous, and actively proliferating; otherwise they resemble those heretofore described. The amount of blood pigment and the number of phagocytes present in some of the glands is very large.

The spleen resembles those previously described very closely, except that there is a very little blood pigment present. Part of this pigment is hæmotoidin and is intracellular, while a part is hæmosiderin, and, situated chiefly outside the cells, is present in large amount.

The bone marrow is the seat of active proliferation and contains large numbers of mature cells, i. e., fully developed amphophiles.

II. Experiment with Hæmolysin.—In the sheep which received hæmolytic serum from Goose 14, as in the other experiment with hæmolytic serum, the leucocytes do not show very characteristic changes (Table VII); their number undergoes two slight but rather prolonged rises and falls. These changes are almost wholly referable to the amphophiles, the lymphocytes varying only slightly throughout the experiment.

The erythrocytes decrease slightly at first and then gradually rise to a number somewhat above normal. This number then suddenly drops from 5,340,000 to 4,280,000 in twelve hours,

1,060,000 erythrocytes, or 19% of the entire number in circulation, being destroyed.

SHEEP 14.—Received 40 cc. serum from Goose 14, on April 23, 1904, at 2 P.M. No erythroblasts were found in any of the preparations of blood. The animal was killed April 28, 3 P.M. At autopsy a large amount of peritoneal fluid is found. Hæmolymp glands are not numerous but are large. The spleen is slightly smaller than normal. The lungs are anæmic. Otherwise the organs are normal; there is no infection.

Histology.—The histological changes resemble very closely those already found after the injection of hæmolytic serum.

The two preceding experiments vary but little from other similar experiments already described, except that heretofore the effect of the hæmolympotoxin has been to inhibit proliferation in the germ centres; in Sheep 12 proliferation is very active.

Under the same conditions, the serum obtained by the injection of emulsion of hæmolymp glands is more hæmolytic than that obtained by the injection of a corresponding amount of erythrocytes; in the first case there is a destruction of 32% of the entire number of erythrocytes in circulation, while in the latter there is a destruction of only 19%.

EXPERIMENTS WITH HEATED HÆMOLYMPHOCYTOTOXIN.

In the following experiment hæmolympocytotoxin was heated to 56° C. for half an hour to destroy the hæmolytic action before injection.

Changes in blood (Table VIII) obtained in this experiment are strikingly like those obtained by Bunting after the injection of lymphotoxin. There is first a leucocytosis of moderate extent due to a combined increase of amphophiles and lymphocytes. Following this there is a still greater leucocytosis and finally a more moderate one. All three leucocytoses are due to a combined rise of both amphophiles and lymphocytes. They differ from those of other experiments by the sharp rise and fall of the lymphocytes which ordinarily rise slowly to a certain height and then gradually decline.

The erythrocytes show, at first, a slight decrease, due to the

fact that the hæmolytic action of the serum was not completely destroyed, but they soon regain their normal height, which they retain throughout the experiment. The large mononuclears vary little; the eosinophile cells show the ordinary preliminary increase and crisis as described above.

SHEEP 4.—Received 30 cc. of serum from Goose 4. at 3 P.M., November 18, 1903. This serum had, previous to injection, been heated to 56° C. for half an hour. The animal was killed 10 A.M., November 22. At autopsy slight oedema is found around the place of inoculation. There is a large amount of peritoneal fluid. The hæmolymp glands are dark in color and apparently not so large nor so numerous as normal. The spleen on section is dark in color, but is otherwise normal. All other organs and tissue are apparently normal; there is no infection.

Histology.—The hæmolymp glands are congested, the sinuses being large and densely crowded with erythrocytes. Germ centres are few in numbers, and when found are small but show fairly active proliferation. Evidences of cell destruction are not marked, although a few pyknotic and vesicular nuclei are to be found. Some blood pigment, probably hæmatoidin, is present. Eosinophile cells are present in great numbers and are both of mononuclear and polynuclear type, but are fewer than in Sheep 3.

Some of the lymph glands, particularly the mesenteric glands, show toxic changes, manifested by more or less wedge-shaped areas of necrosis (Fig. 1) extending into the lymph glands with their bases toward the periphery. These areas appear as granular eosin-staining masses around which in some cases there is a beginning attempt at sequestration by leucocytes. Phagocytes containing fragmented nuclei are very abundant. The germ centres, while not large, show fairly active proliferative changes. Some blood pigment, probably hæmatoidin, is present, and is contained entirely in large phagocytes. There is an enormous increase in eosinophiles, both mononuclear and polynuclear.

The spleen is congested and shows signs of degeneration, pyknotic and fragmented nuclei being present. There is a large increase of eosinophiles. A small amount of blood pigment is present.

The changes in the bone marrow consist chiefly in a marked increase in number of the myelocytes. Mature amphophiles are also numerous, eosinophiles are decreased in numbers, and lymphocytes are numerous. Throughout all the tissues examined there is a marked proliferation of endothelium.

The kidneys in this animal, in contrast to the others cited, show the presence of iron situated in the tubules and demonstrable by the appropriate reaction.

Histological examination shows that the amphophile leucocytosis is due to the proliferation of cells in the bone marrow antecedent to the amphophiles, while the increase of lymphocytes is due almost entirely to the existence of preformed cells, since proliferation in the germ centres is not marked.

A second experiment was conducted in exactly the same way as the preceding; the serum of a goose which had been immunized to hæmolymph glands being heated to 56° C. for a period of half an hour. It was expected that at the expiration of this time the hæmolytic property, at least that due to immunization with injected erythrocytes, would be destroyed.

Changes in the blood (Table IX) following the injection of this serum into the sheep resemble in only one point those obtained in the preceding experiment; there is at first leucopenia followed by a slight, gradual leucocytosis, after which there is a slight drop, another slight rise, and then a gradual return to normal. Changes affecting the entire number of leucocytes are closely followed by those of the lymphocytes, the amphophiles being so few in numbers that they have little effect upon these changes.

The erythrocytes undergo a slight preliminary rise of 700,000 and then a sharp fall of two million, after which they gradually return to normal. The eosinophiles slowly but markedly increase until the sixty-fourth hour, when the so-called crisis occurs. The large mononucleus and mast cells show no noteworthy alteration.

SHEEP 19.—Received 45 cc. of serum from Goose 19 on May 16, 1904, at 1 P.M. The serum previous to injection had been heated to 56° C. for half an hour. Sheep 19 was killed at 10 A.M., May 21. There was a moderate amount of peritoneal fluid. The hæmolymph glands were rather large and numerous. All the organs were apparently normal. Bone marrow was light in color. There was no infection.

Histology.—The hæmolymph glands are congested, the sinuses being large and densely crowded with erythrocytes. The germ centres are large and actively proliferating. There is, moreover, a slight general hyperplasia of lymphoid cells. There is little evidence of degeneration. Endothelial proliferation is marked. Large numbers of phagocytes containing entire corpuscles and some containing blood pigment, hæmatoidin, are to be seen. There is an enormous increase of eosinophiles.

The germ centres in the lymph glands are also large and actively proliferating.

In this case no necrotic areas, similar to those observed in the previous experiment, are found. Degenerative changes are fairly numerous, vacuolar, vesicular, and some pyknotic nuclei being observed.

Large numbers of phagocytes containing blood pigment, hæmatoidin, are to be seen. Eosinophiles are increased in numbers. The spleen is normal,

except for the presence of a large amount of blood pigment which, situated outside of the cells, is for the most part hæmosiderin. Mitosis in the germ centres is active, and there is a rather marked proliferation of the endothelium. Eosinophiles are somewhat increased.

In contrast to the previous experiment, the kidney tubules do not show the presence of iron.

The bone marrow seems to be little affected, and is practically normal. There is, however, some increase of erythrocytes and of eosinophiles. The presence of large numbers of mature amphophiles is noteworthy when it is recalled that few were present in the circulation.

This experiment differs markedly from the one preceding it, the changes not only in the blood but in the tissues being different.

These experiments suggest, in accordance with the statement of Pearce, that necrosis is not a specific action of the serum, but is due to its erythrolytic and agglutinative properties.

The marked hæmolysis which occurred in the last experiment is, however, of great importance, for it tends to show the presence of some hæmolytic factor, which is resistant to heat and not due to immunization with erythrocytes alone.

EXPERIMENTS WITH HETEROHÆMOLYMPHOTOXIN.

In the following experiment a hæmolympphotoxin for the sheep was prepared by the usual method, and was then injected into a rabbit in order to determine if a hæmolympphotoxin active for one animal would have the same action in an animal of a different species or if its action would be peculiar to the species for which it was prepared. Following this injection (Table X) there is an extremely marked preliminary leucopenia showing that the peritoneal irritation has been extreme. This leucopenia is followed by a well-developed leucocytosis, which is in turn followed by a decided drop in the number of leucocytes, interrupted by one slight rise; the number of leucocytes then gradually returns to normal. The amphophiles rise steadily for twenty hours, after which they as steadily decrease, rising to normal just before death. The preliminary decrease of lymphocytes is well-marked and of long duration; these cells do not begin to increase in number until the twentieth hour, after which they very closely follow the total number of leucocytes.

It would appear, then, as if proliferation in the lymphocyte-forming organs had received a check, or that amoeboid movement of the fully developed cells in the lymph glands and spleen was slower or more difficult than usual.

The eosinophiles follow the usual course observed after the injection of hæmolymphotoxin, decreasing at first and then rising until the forty-fourth hour, when the so-called crisis occurs.

Changes undergone by the erythrocytes are of great interest since they give evidence of very marked hæmolytic action. There is at first a sharp rise of about one million, and then in the next five hours a decided drop of over three millions. Subsequently the number fluctuates as if the destruction at times overcame the supply, but steadily returns to normal.

RABBIT 1.—Received 10 cc. of serum from Goose 12 at 12 noon, April 23, 1904. Killed, 1 P.M., April 28. At autopsy the organs were found to be normal; there was no infection. No hæmolymph glands were found at autopsy.

Histology.—The germ centres in the lymph glands are fairly well developed, and proliferation is active. Mitotic figures are fairly numerous throughout the gland. Degenerative changes also are very abundant, many large vacuolar, vesicular, pyknotic, and fragmenting nuclei being present. Endothelial proliferation is also very marked, and many large endothelial phagocytes contain particles of pigment, probably hæmatoidin, while some contain fragments of nuclei. Eosinophiles are greatly increased in numbers.

Proliferative changes in the spleen are less evident than in the lymph glands, but there is very marked endothelial proliferation. The blood pigment here present in rather large amounts is hæmatoidin. Eosinophiles are markedly increased in numbers.

The cellular elements throughout the bone marrow are undergoing proliferation; the endothelium is also the seat of proliferation.

Comparison of the histological changes just described and those produced by the injection of hæmolymphotoxin into a sheep shows that they are almost identical.

Difference in the changes affecting the blood and perhaps the slightly more toxic effect which the serum seems to have on the rabbit may be explained by the fact that a greater amount of serum per kilo of body weight was injected into the rabbit. Moreover, the hæmolytic effect on the rabbit is very marked, a destruction of 43% of the total number of erythrocytes in circulation having occurred in five hours.

The eosinophiles of the rabbit, like those of the animal for which the hæmolymphotoxin was prepared, are increased greatly in numbers. It is probable, then, that the toxin produced by the injection of emulsions of hæmolymph glands into an intermediary animal is heterotoxic, the changes in the two species employed being the same in every respect.

SUMMARY OF EXPERIMENTS WITH HÆMOLYMPHOCYTOTOXIC SERUM.

After the injection of the serum of an animal immunized against hæmolymph glands there is a series of changes which may be classified in several groups.

I. *Changes Due to Hæmolytic Properties.*—The hæmolytic properties of the so-called hæmolymphotoxin are very marked and their effects are observable not only in the blood counts, but also in the tissues. Its hæmolytic power is in fact stronger than that of the serum obtained when the same number of corpuscles are injected into the intermediary animal as are contained in the emulsion of hæmolymph glands employed. This fact is demonstrated by the following table in which parallel experiments are compared:

(a) Normal serum caused an increase of 1,000,000 erythrocytes.

(b) 1-Hæmolytic serum caused the destruction of 3,000,000 erythrocytes, or 28% of the entire number in circulation.

2-Hæmolymphotoxic serum caused the destruction of 4,392,000 erythrocytes, or 47% of erythrocytes in circulation.

(c) 1-Hæmolytic serum caused the destruction of 1,060,000, or 19% of erythrocytes in circulation.

2-Hæmolymphotoxic serum destroyed 1,560,000, or 32% of erythrocytes in circulation.

There is, therefore, a difference in one case of 19% and in the other of 13% between parallel experiments. Taking into consideration the fact that in one case the goose serum may have had a stronger inherent tendency to destroy erythrocytes than in the other and the possible percentage of error, it is still impossible to explain the difference between the two experiments.

Moreover, when the serum was heated to a temperature sufficient to destroy all hæmolytic action in a serum produced by the injection of erythrocytes alone, there was still considerable destruction of erythrocytes after injection into the sheep. Furthermore, on injection of the toxin into an animal of a different species the hæmolytic effect was very marked—more marked than would usually follow the injection of hæmolytic serum produced with erythrocytes alone.

Taking all these facts into consideration, it seems probable that the hæmolymphotoxin has some hæmolytic action not due to the reaction of the goose to erythrocytes alone.

II. *Changes Due to Lymphotoxic Properties.*—Certain of the changes produced by the injection of hæmolymphotoxin are referable to the immunization of the goose against the lymphoid tissue in the hæmolymph glands.

In one experiment there is apparently an inhibition of proliferation in the germ centres, while in the other two proliferation is marked. This occurrence of proliferation agrees with results generally obtained after injection of lymphotoxin. While proliferation is not necessarily due to the direct action of the toxin, in most instances at least no inhibition of proliferation is caused by hæmolymphotoxin.

The changes affecting the leucocytes of the blood and to a certain degree the proliferative changes in the blood-forming organs are due to the peritoneal irritation following the injection of the toxin; to a certain extent they are referable to the direct effect of the toxin.

The degenerative changes which occur are, however, due to the lymphotoxic action, and those following the injection of hæmolymphotoxin are in all respects like those following the use of lymphotoxin.

III. *Changes Due to Endotheliotoxic or Phagocytotoxic Properties.*—In order to demonstrate the presence of an endotheliotoxin or phagocytotoxin a comparison may be made of the number of phagocytes present in the spleen and lymph glands after the injection of hæmolymphotoxin, lymphotoxin, and hæmolysin.

Such a toxin is doubtless present in the hæmolymphotoxic serum employed for it is readily seen, even when a low-power objective is used, that the number of phagocytes present after the injection of hæmolymphotoxin is much greater than after the injection of hæmolysin. That their presence, moreover, is not secondary to the destruction of erythrocytes is shown by the fact that the number of phagocytes found after the injection of hæmolymphotoxin is greater absolutely than the number present after the injection of hæmolysin, while with hæmolymphotoxin the phagocytes are increased out of proportion to the number of red blood cells destroyed. There is further proof that this accumulation of phagocytes is not secondary to destruction of erythrocytes; Warthin (9) has described similar changes in hæmolymph glands after removal of the spleen.

It is probable, therefore, that an endotheliotoxin or phagocytotoxin is present in the serum.

IV. *Changes Due to Hæmolymphotoxic Properties.*—Is it necessary to assume the existence of a specific hæmolymphotoxin in order to explain the results obtained?

In the first place, there is produced a certain amount of hæmolysis which is not due alone to the erythrocytes injected into the goose.

One theory regarding the action of hæmolymph glands is that they are hæmolytic. Such hæmolysis may be explained in two ways: (1) it is due to a ferment, secreted by phagocytes; (2) the phagocytes take up and remove corpuscles bodily from the circulation.

If the first hypothesis is correct, it is evident that in immunizing the goose with emulsions of hæmolymph glands this destructive action upon erythrocytes must be overcome as well as the native resistance of the erythrocytes themselves; so that, in the end, the goose serum would become more destructive to erythrocytes than if the erythrocytes contained in the emulsions of hæmolymph glands had been alone injected.

The changes which occur in the sheep injected with hæmolymphotoxic serum are referable to substances produced during the reaction of the goose to the tissues injected into it, namely,

lymphoid tissue, endothelium, etc., and to the peritoneal irritation in the sheep due to the injection of the serum; hence it is unnecessary to assume the existence of a specific hæmolymphotoxin.

V. *Heterotoxic Properties*.—The serum obtained after the injection of emulsions of hæmolymph glands into an intermediary animal is heterotoxic, for when it is injected into an animal of a different species every detail of the process is duplicated and consequently it is not specific for the primary species alone.

CONCLUSIONS REGARDING HÆMOLYMPHOTOXIN.

(1) The toxin produced by the injection of emulsions of hæmolymph nodes from one species into an intermediary animal is complex in composition, having lymphotoxic, endotheliotoxic or phagocytotoxic and hæmolytic properties.

(2) These toxins when so obtained are heterotoxic, affecting other species besides that one which was used for the immunization of the intermediary animal.

(3) There is no specific hæmolymphotoxin.

(4) The chief function of the hæmolymph glands is hæmolysis; they remove partially broken-down erythrocytes from the circulation.

(5) Hæmolysis is probably brought about by a ferment produced in the hæmolymph node.

(6) This ferment is aided by phagocytes which remove the debris from the glands.

(7) The ferment is probably produced by the endothelium.

(8) In this connection it is desirable to emphasize the theory that hæmolymph glands have a part in the production of eosinophiles, the granules arising from products of disintegration of red cells. Such material is taken up by mononuclear leucocytes the nuclei of which become polymorphous. In almost every experiment there has been some destruction of erythrocytes and there has been an increase of eosinophiles in the blood and lymphoid structures in proportion to this destruction. Furthermore, when, in making special stains for eosinophiles, the cells were overstained with eosin and part of the eosin was afterward

removed, the phagocytes containing particles of erythrocytes tended to hold the stain longer than either ordinary phagocytes or normal erythrocytes.

After the preceding conclusions concerning hæmolymphotoxin have been reached, it becomes necessary to study carefully by the same methods other so-called cytotoxins in order to ascertain to what extent the changes produced by them vary from those already described and what degree of specificity each possesses.

EXPERIMENTS WITH ENDOTHELIOTOXIN.

So far as can be ascertained, no attempt has heretofore been made to produce a pure endotheliotoxin. In order to produce such a toxin the aortas of sheep were removed and the endothelium, under as nearly sterile conditions as possible, was scraped off and made into an emulsion in sterile physiological salt solution. Great care was necessary to avoid the removal of connective tissue and the entrance of blood into the emulsion.

The toxin produced by the injection of this emulsion into a goose gave, on being injected into a sheep, a rather typical blood picture (Table XI). There is at first a rather sharp leucopenia, followed by a leucocytosis, reaching its summit at the end of about twenty hours. The number of leucocytes then gradually decreases and again rises, producing a leucocytosis which, of greater height than the first, keeps its elevation for about twenty-five hours, the number then returning to normal. After the so-called "anticipatory" leucocytosis the changes in the total number of leucocytes are very closely followed by those of the lymphocytes, the amphophiles after the first rise markedly decreasing and then very gradually rising to a level somewhat above normal, to decrease again sharply.

Changes in the number of erythrocytes are noteworthy; from the first, with the exception of a slight rise at the end of the sixty-sixth hour, it gradually yet steadily decreases, falling from 5,160,000 before injection to 2,760,000 at time of death—a destruction of 2,400,000 corpuscles or over 46% of entire number of erythrocytes in circulation.

SHEEP 15.—Received 40 cc. of serum from Goose 15 at 1.30 P.M., April 9, 19. One nucleated red blood corpuscle was seen in counting five hundred cells on April 10, at 9.30 P.M. The animal was killed April 15, 10.30 A.M. At autopsy the entire body is anæmic, though fairly well nourished. A large amount of intraperitoneal fluid is present. The hæmolymph glands are numerous and hyperplastic. The liver and kidneys are normal. Spleen is slightly smaller than normal. The heart and lungs are normal. The bone marrow is fairly red. There is no infection.

Histology.—The germ centres in the hæmolymph glands are more numerous and larger than in any experiment heretofore examined. Proliferation in the germ centres is exceedingly active. Many large phagocytes filled with blood pigment or with entire red blood corpuscles are to be seen. Endothelial proliferation is very marked. Evidence of degeneration is rarely found. Eosinophiles are somewhat increased in numbers.

In the lymph glands, as well as in the hæmolymph glands, the germ centres are large, numerous, and show signs of very active proliferation. Proliferation of endothelium is also very marked, and large numbers of endothelial phagocytes containing blood pigment, probably hæmatoidin, are to be seen. On the other hand, degenerative changes are here very numerous, many vesicular, some vacuolar, and a few pyknotic nuclei being found. Eosinophiles are normal.

The spleen is slightly smaller than normal. The germ centres in the spleen are large and are the seat of active proliferation. Endothelial cells have undergone proliferation and many endothelial phagocytes containing blood pigment, probably in this organ also hæmatoidin, are to be seen. Degenerative changes are marked.

Proliferative changes in the bone marrow are widespread, every type of cell being involved in the process, which affects particularly the endothelium, so that large endothelial cells may be seen in every stage of multiplication. The myelocytes and erythroblasts are also greatly increased in number. Large numbers of mature cells such as amphophiles are present.

The liver shows a slight fatty degeneration and the kidney a slight amount of cloudy swelling.

The results obtained in the preceding experiment are important because they demonstrate the occurrence of marked changes not only in the blood but in the blood-forming organs. The marked and steady decrease in the number of erythrocytes after the injection of the toxin indicates that it is possible for the endothelium alone, through its phagocytic tendencies, to cause a profound and perhaps lasting anæmia. It cannot, however, be maintained that this destruction of erythrocytes is due to phagocytic action alone, for the histological changes will not justify such a statement. It is not improbable that a large part of it is due to the secretion of some ferment, such as that present in

the hæmolymp glands; it destroys the erythrocytes and causes their removal from the circulation.

EXPERIMENTS WITH HEPATOTOXIN.

In 1900, Delezenne (11) obtained a *serum antihepatique* by injecting emulsions of the liver of dogs into rabbits and ducks. A strongly toxic serum causing death in doses of 4 cc. per kilo of body weight was obtained. At autopsy, the liver was yellow, friable, and shrivelled, the animal having apparently died with a condition resembling that produced by phosphorus. By microscopical examination the cells were found to be markedly degenerated. In certain areas the lesion was more advanced and the protoplasm of the cells had in great part disappeared. The blood-vessels were dilated and capillaries filled with blood separated the rows of degenerated hepatic cells. There was an infiltration of leucocytes throughout the lobules. When the process was less acute, the animal lived from five to fifteen days, and marked fatty degeneration of the liver alone was noted.

Pearce (12) produced hepatotoxins with emulsions made from liver tissue washed entirely free of blood and with unwashed liver. After injection of hepatotoxins prepared with unwashed liver he found no changes in the hepatic cells which could be ascribed to their specific action. With sera from washed livers he found increased granulation of the liver cells, with slight, fatty transformation. Necrotic areas were found in only one experiment in which a large dose of strongly hæmagglutinative serum was employed; he believes that these necrotic areas are due to thrombi formed by agglutinated red blood cells.

In the experiment to be described (Table XII) there are no characteristic changes in the blood. There is a sharp preliminary leucocytosis, followed by a step-like decrease in the number of leucocytes; subsequently there are two minor rises. The first leucocytosis is altogether due to an increase in amphophiles; the subsequent leucocytoses are due to an increase of both amphophiles and lymphocytes.

The eosinophiles undergo a preliminary decrease, followed by a gradual rise and fall.

The erythrocytes exhibit rapid preliminary increase, followed by a rather sharp and well-marked fall, after which there is a return to normal, again followed by a very gradual yet distinct decrease.

SHEEP 16.—Received 35 cc. of hepatotoxic serum from Goose 16 at 1 P.M., April 5, 1904. Killed, 11 A.M., April 11. At autopsy the hæmolymph glands are found to be few in number. The spleen is slightly smaller than normal. There is an elevated white nodule, 5 x 5 x 5 mm. on the liver. The liver is light in color and apparently contains fat. The spleen is dark in color. The other organs are normal. There is no infection.

Histology.—The germ centres in the hæmolymph glands are large, and are the seat of active though not excessive proliferation. There is little evidence of degeneration, although some pyknotic and vesicular nuclei are to be seen. There is some proliferation of endothelium. Eosinophiles are decreased in number.

The lymph glands show no changes beyond a slight increase of proliferation in the germ centres and endothelium, and the presence of a considerable amount of blood pigment, probably hæmatoidin, contained for the most part within endothelial phagocytes.

The spleen is slightly smaller than normal and somewhat congested. There is increased proliferation both in the germ centres and in the endothelium. Some blood pigment is present, but it is entirely hæmatoidin, for no reaction for iron could be obtained.

The bone marrow is almost wholly normal, only a slight increase of myelocytes and erythroblasts being apparent.

The elevated white area on the liver noted at autopsy proved to be an encysted animal parasite. The liver is the seat of fairly advanced fatty degeneration. The kidney shows only a very slight cloudy swelling.

Changes in the blood, as before stated, are limited to the rather marked hæmolysis. Although an attempt was made to wash the blood from the liver before injecting it into the goose it was impossible to remove the blood entirely, so that hæmolysis is probably for the most part due to this factor. The histological changes are not characteristic; changes in the blood-forming organs are insignificant and the fatty change in the liver, while confirming the results obtained by Delezenne, is not so marked as that produced by some other cytotoxins. Nearly every cytotoxin causes at least a slight fatty degeneration of the liver. We may say, then, that the hepatotoxins have no specific action.

EXPERIMENTS WITH SPLENOTOXIN.

Flexner (3) found after the injection of splenotoxin that the chief changes were in the lymph glands and spleen. In the lymph glands the germ centres were large and the cells had undergone degeneration, but few mitotic figures were present. In the spleen there was wide distension of the blood sinuses, slight general hyperplasia of splenic cells, and marked evidences of degeneration.

Changes in the blood (Table XIII) obtained in the following experiment are peculiar. There is at first a rather rapid rise and fall of the number of leucocytes, soon followed by a slighter rise and fall; there is then a period of about forty-eight hours during which the leucocytes vary but little; a marked increase in number occurs and is followed by a gradual decrease. The first increase is entirely due to an amphophile leucocytosis, there being at the same time the usual lymphopenia. The second increase is referable to the lymphocytes, while the third is again due to amphophiles.

The eosinophiles decrease at first and then gradually rise until the so-called crisis occurs at the end of thirty-six hours.

Changes affecting the erythrocytes are characteristic; hæmolytic causes the destruction of only 15% of the erythrocytes present in the circulating blood.

SHEEP 18.—Received 30 cc. of splenotoxic serum from Goose 18 at 1 P.M., April 14, 1904. Killed, April 21, 1.30 P.M.

Autopsy.—Hæmolymph glands are small but numerous. The spleen is smaller than normal. The kidneys are normal. The liver is light in color. The bone marrow is anæmic. Moderate amount of peritoneal fluid is present. The heart and lungs are normal. There is no infection.

Histology.—The hæmolymph glands are somewhat congested. Germ centres are few in number, and there is little proliferation of cells except in the endothelium. Degenerative changes are very numerous, and many vesicular and pyknotic nuclei are to be seen. Eosinophiles are decreased in numbers.

The germ centres in the lymph glands are sparsely scattered, but those which are found are large; they show many degenerative changes, the nuclei of the cells being swollen and vacuolar.

The spleen is small. There is a slight tendency to general hyperplasia of the lymphadenoid tissue, but more conspicuous is the marked degeneration, characterized by the presence of pyknotic and fragmented nuclei. In the

haemolymph glands, lymph glands, and spleen numerous endothelial phagocytes are found containing blood pigment, probably hæmatoidin, and nuclear debris.

The bone marrow contains much fat, and few changes from the normal are to be observed, except perhaps proliferation of endothelium.

The liver shows advanced fatty degeneration, the liver cells being occupied by large globules of fat. The distribution of fat is fairly uniform throughout the lobules.

The kidneys show slight cloudy swelling.

The changes observed in this experiment resemble very closely those described by Flexner. The toxin apparently has a specific effect upon the spleen, causing extensive degenerative changes; these changes, however, do not differ so much from those produced by other toxins that we are justified in assuming that the difference is one of kind and not of degree. The fatty degeneration of the liver is particularly noteworthy since it is greater in extent than that found after the injection of hepatotoxin, and therefore makes still more doubtful the specificity of that toxin.

EXPERIMENTS WITH NEPHROTOXIN.

In a recent article by Pearce (12) the subject of nephrotoxins is so carefully reviewed that it will be necessary here merely to describe their action upon the blood and blood-forming organs.

Changes in the number of leucocytes (Table XIV) are characterized by a preliminary leucopenia which is of long duration. This leucopenia is followed by a well-marked rise and fall and later by two lesser rises. The behavior of the leucocytes seems to depend upon the lymphocytes for, saving a slight rise in the number of amphophiles during the first leucocytosis, the changes of the total number of leucocytes and of lymphocytes are very similar.

The erythrocytes show at first a slight increase in number, and then a gradual decrease, showing that the serum is somewhat hæmolytic in spite of the fact that a large part of the red blood corpuscles have been washed out of the emulsion before it has been injected into the goose.

SHEEP 17.—Received 40 cc. of nephrotoxic serum from Goose 17 at 2 P.M., April 1, 1904. Killed, April 7, 11 A.M.

Autopsy.—The hæmolymph glands are dark in color. The liver bleeds freely on section. There is well-marked congestion of the medullary portion of the kidney. All other organs are normal; there is no infection.

Histology.—The hæmolymph glands are fairly normal, having well-developed germ centres, which are the seat of active proliferation, and are in all respects normal. Endothelial phagocytes are somewhat more abundant than normally. They contain particles of blood pigment, probably hæmatoidin, and entire erythrocytes. Eosinophiles are normal in number. There is an increased proliferation of endothelium.

The germ centres in the lymph glands are large and actively proliferating. In the mesenteric glands in particular there is a considerable amount of blood pigment, probably hæmatoidin, contained in large phagocytes. There is considerable proliferation of endothelium shown by the presence of large, free, mononuclear endothelial cells, some of which are the phagocytes above described. The eosinophiles are slightly increased in numbers.

The spleen is little affected by the injection of nephrotoxin, and is normal save for a slightly increased proliferation of the endothelium. There is present a small quantity of free blood pigment, hæmosiderin.

The bone marrow is little altered. All the cellular elements, particularly the eosinophile cells, are undergoing proliferation, which is slightly more active than usual.

The congestion of the medullary part of the kidney, noted macroscopically, is in places so marked that the lumina of the tubules are encroached upon. There are a few hyaline casts. The cells of the tubules furthermore show some cloudy swelling and desquamation, which is most marked in the cortex, where some of the tubules are entirely filled with debris.

The changes produced by nephrotoxin in the blood and blood-forming organs are insignificant; those changes which occur, namely, proliferation in the germ centres and proliferation of the endothelium, are secondary, the one to the peritoneal irritation, and the other to destruction of erythrocytes.

The changes in the kidneys, however, are well marked and characterize the action of the serum.

EXPERIMENTS WITH LYMPHOTOXIN.

Peritoneal irritation following the injection of lymphotoxin prepared by the method already described is doubtless severe since there is a marked preliminary leucopenia (Table XV). This leucopenia is soon followed by a rather pronounced increase in the number of leucocytes, the bone marrow apparently responding to the demand upon it. The available supply having been exhausted, the leucocytes decrease and then rise again more

slowly yet to a greater height; this increase of the total number is due to an increase both of amphophiles and lymphocytes. The slow response of the lymphocytes to a demand upon them is evident since the preliminary lymphopenia is marked and of considerable duration, the increase of lymphocytes being gradual until the final leucocytosis which is almost entirely due to lymphocytosis.

The erythrocytes increase at first and then gradually return to normal. The eosinophiles and large mononuclears show no noteworthy change.

SHEEP 11.—Received 30 cc. of lymphotoxic serum from Goose 11, March 25, 1904, at 2.30 P.M. The animal was killed March 31, at 10 A.M.

Autopsy.—There is a small amount of peritoneal fluid. The hæmolymph glands are small and few in numbers. The left kidney is slightly smaller than normal. The spleen is somewhat small. Lymph glands are numerous and rather hyperplastic. There is a considerable amount of pericardial fluid. Little fat is present throughout the body. The liver is normal. There is no infection.

Histology.—The hæmolymph glands are considerably congested and contain only a small amount of lymphoid tissue. The few germ centres which are present are, however, large and show active proliferative changes. There is also considerable proliferation of endothelium. The eosinophiles are few in number, being considerably less abundant than usual. There is some pyknosis.

The germ centres in the lymph glands are numerous and large, showing many mitotic figures. There is also evident proliferation of endothelium, and large numbers of endothelial phagocytes containing blood pigment, probably hæmatoidin, are present. Evidences of degeneration are limited to the presence of pyknotic, vesicular, and a few fragmented nuclei; there are no necrotic areas similar to those found by Bunting (4) after the injection of heated hæmolymphotoxic serum. The eosinophiles are decreased in numbers.

The spleen is somewhat atrophic. The germ centres are large, numerous, and the seat of active proliferation. A considerable amount of both extra-cellular and intracellular pigments is present, and is in great part, if not entirely, hæmosiderin. There is little evidence of degeneration. Eosinophiles are decreased in number.

The cellular elements of the bone marrow are markedly depleted. Those which are present are, however, undergoing marked proliferation. The myelocytes exhibit most active proliferation, the erythroblasts stand next in order, and the eosinophiles show the fewest mitotic figures. Fully-developed cells are few in numbers, but a few amphophiles are present.

The changes just described differ markedly from those obtained by Bunting, and resemble more closely those observed

by Flexner. They do not have a specific character, nor do they vary greatly from the changes observed after the injection of other cytotoxins.

SUMMARY OF CYTOTOXIC EXPERIMENTS.

Are the cytotoxins specific—that is, do they individually produce characteristic changes or do the changes which they produce differ in degree alone?

(1) Changes in the blood observed in the preceding experiments are in no instance characteristic and may be explained by the action on the blood and blood-forming organs, of the peritoneal irritation produced by the injection of the toxin. These changes do not vary sufficiently from those obtained by the injection of salt solution and of normal serum to justify the assumption that they show, in any case, anything specific, particularly when we take into consideration the different conditions under which the animals may have been at time of injection.

(2) Histologically, certain differences are evident, but again all of these are of degree and not of kind. For instance, fatty degeneration of the liver was found after injection of hepatotoxin, but in almost every case examined there was slight fatty degeneration, while in the case of splenotoxin the fatty change was much greater than after hepatotoxin.

In every experiment almost the same changes were observed; they may be classified as follows:

Hæmolymp Glands.—Proliferation in the germ centres and endothelium; large numbers of endothelial phagocytes containing blood pigment, probably hæmatoidin; rather marked degenerative changes.

Spleen.—Proliferation in the germ centres and endothelium; some phagocytes containing blood pigment and usually more or less free iron-containing pigment, probably hæmosiderin.

Bone Marrow.—Proliferation of all elements.

Kidney.—Slight cloudy swelling.

Liver.—Slight fatty degeneration.

Throughout the entire series of injections these changes are

to be observed and they vary merely in degree. All the cytotoxic sera are therefore hæmolytic, endotheliolytic, lymphotoxic, myelotoxic, etc.

When we take into consideration the severity of the changes which occur, the various sera may be arranged in the following order:

- (a) Hæmolymphotoxin,
- (b) Splenotoxin,
- (c) Lymphotoxin,
- (d) Endotheliotoxin,
- (e) Nephrotoxin,
- (f) Hepatotoxin.

It is readily seen that the above order takes into consideration the amount of hæmolysis produced by each serum. In the case of the spleen and the hæmolymph glands it is practically impossible to remove this factor, and in making the above classification the other factors have been given greater importance than this one. We have, then, in every case, a serum which causes a certain series of changes which always vary as to degree but not as to kind.

(3) The nephrotoxin perhaps comes nearest to being specific, since macroscopically and microscopically marked congestion of the medullary portion of the kidney and pronounced cloudy swelling in the cortex were observed. Similar changes of the same severity were found with no other serum, and it appears that the renal toxin had a specific, perhaps merely irritating, action on the kidney itself.

(4) It seems, furthermore, that the hæmolymph glands play some important part in the production of eosinophile cells, for in no case was there such a marked increase of these cells as after the injection of hæmolymphotoxin; indeed, after most of the toxins used, the number of eosinophile cells was found decreased.

CONCLUSIONS REGARDING CYTOTOXINS.

- (1) The cytotoxins are not specific.
- (2) The changes occurring after the injection of different cytotoxins are similar and do not vary in kind.

(3) The changes do, however, vary in degree in the following order, the one having the most marked effect being placed first, etc.:

- (a) Hæmolymphotoxin,
- (b) Splenotoxin,
- (c) Lymphotoxin,
- (d) Endotheliotoxin,
- (e) Nephrotoxin,
- (f) Hepatotoxin.

(4) Nephrotoxin causes the most specific change.

(5) The hæmolymph glands play some important part in the production of eosinophiles. The hæmolytic action of hæmolymphotoxic serum is greater than that of other cytotoxic sera.

(6) The histological changes which are observed in every case are secondary to the hæmolytic factor present in all cytotoxic sera.

In conclusion I wish to express my thanks to Dr. Warthin, in whose laboratory and under whose directions these experiments were conducted, for his aid and encouragement in the work.

DESCRIPTION OF PLATES.

PLATE IV.

Fig. 1.—Area of necrosis in lymph gland of Sheep 4 after injection of heated hæmolymphocytotoxin. Stained with hæmatoxylin and eosin.

Fig. 2.—Increase of eosinophile cells in hæmolymph gland of Sheep 3 after injection of hæmolymphocytotoxin. Mononuclear, polymorphonuclear, and polynuclear eosinophiles are found in the lymphadenoid tissue of the hæmolymph gland. Stained with hæmatoxylin and eosin.

PLATE V.

Fig. 3.—Hæmatoidin in lymph gland of Sheep 3 after injection of hæmolymphocytotoxic serum. Stained with hæmatoxylin and eosin.

Fig. 4.—Iron-containing pigment found in spleen after injection of hæmolymphocytotoxin, demonstrated by the Prussian-blue reaction. Counter-stained with borax carmine.

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TABLE I.

Time.	Feb. 14, 10 A.M. Before injection.		Feb. 14, 2.30 P.M. 3 1/2 hours after injection.		Feb. 14, 10.30 P.M.		Feb. 15, 8.30 A.M.		Feb. 15, 8.30 P.M.		Feb. 16, 8.30 A.M.		Feb. 16, 8.30 P.M.		Feb. 17, 8.30 A.M.	
	Per cent.	Total.	Per cent.	Total.	Per cent.	Total.	Per cent.	Total.	Per cent.	Total.	Per cent.	Total.	Per cent.	Total.	Per cent.	Total.
Lymphocytes.....	32.2	4,079	26.4	3,627	31.5	5,065	36.7	4,646	37.5	5,108	33.6	5,080	34.	4,821	30.8	4,035
Large Mononuclears.....	7.2	912	6.5	893	5.8	933	7.3	924	8.6	1,171	8.1	1,225	6.4	908	8.	1,048
Amphophiles.....	52.6	6,662	63.	8,656	58.7	9,439	52.1	6,596	44.1	6,006	52.1	7,878	51.8	7,345	52.	6,812
Eosinophiles.....	8.	1,013	3.9	536	4.	643	3.7	469	9.8	1,335	6.	907	7.8	1,106	8.4	1,100
Mast Cells.....	0	0	.2	28	0	0	.2	25	0	0	.2	30	0	0	.8	105
Total Leucocytes.....	100	12,666	100	13,740	100	16,080	100	12,660	100	13,620	100	15,120	100	14,180	100	13,100
Erythrocytes.....	7,500,000		7,000,000		7,500,000		7,000,000		6,800,000		6,800,000		7,200,000		7,200,000	

Differential count after injection of cold sterile physiological salt solution.

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TABLE II.

Time.	Dec. 1, 1 P.M. Before injection.		Dec. 1, 7 P.M. 4 hrs. after injection.		Dec. 1, 12 P.M.		Dec. 2, 9 A.M.		Dec. 2, 4 P.M.		Dec. 3, 8 A.M.		Dec. 3, 8 P.M.		Dec. 4, 9 A.M.		Dec. 5, 9 A.M.		Dec. 6, 9 A.M.		Dec. 7, 9 A.M.	
	Per cent.	Total.	Per cent.	Total.	Per cent.	Total.	Per cent.	Total.	Per cent.	Total.	Per cent.	Total.	Per cent.	Total.	Per cent.	Total.	Per cent.	Total.	Per cent.	Total.	Per cent.	Total.
Lymphocytes.....	4.735	6,504	4.791	6,537	4.791	6,537	5.137	7,023	4.833	7,023	7.023	7,023	7.058	7,058	7.156	7,156	5.256	5,256	5.558	5,558	4.725	4,725
Large Mononuclears.....	4.10	537	285	537	285	537	475	1,045	1.076	1,045	1,045	1,045	919	919	1,097	1,097	546	854	854	854	824	824
Amphophiles.....	4.935	3,606	6,689	3,606	6,689	13,911	13,911	7,023	13,520	7,023	7,023	7,023	4,178	4,178	5,128	5,128	2,845	2,694	2,694	2,694	2,974	2,974
Eosinophiles.....	1.40	86	95	86	95	178	178	1,209	117	1,209	1,209	1,209	1,338	1,338	1,364	1,364	754	894	894	894	919	919
Mast Cells.....	0	0	0	0	0	59	59	33	20	33	33	33	27	27	75	75	19	50	50	50	28	28
Total Leucocytes.....	9.320	10,733	11,860	10,733	11,860	19,760	19,760	16,333	19,566	16,333	16,333	16,333	13,520	13,520	14,820	14,820	9,420	10,050	10,050	10,050	9,470	9,470
Erythrocytes.....	12,000,000		12,800,000		13,340,000		14,240,000		13,640,000		13,620,000		13,640,000		13,380,000		14,100,000		13,200,000		14,520,000	

Differential count after injection of normal serum of the goose (Sheep 2).

TABLE III.

Time.	Feb. 9, 9 A.M. Before injection.	Feb. 9, 3 P.M. 3½ hrs. after injection.	Feb. 9, 10.30 P.M.	Feb. 10, 8.30 A.M.	Feb. 10, 7.30 P.M.	Feb. 11, 9 A.M.	Feb. 11, 5 P.M.	Feb. 12, 9 A.M.	Feb. 13, 9 A.M.	Feb. 14, 9 A.M.	Feb. 15, 9 A.M.
Lymphocytes.....	3,009	1,804	2,572	3,151	2,313	2,809	2,466	2,784	3,919	4,702	4,605
Large Mononuclears..	734	649	625	491	453	551	408	545	601	789	556
Amphophiles.....	1,798	2,921	3,895	2,748	1,487	1,378	1,188	1,891	2,133	2,385	2,271
Eosinophiles.....	563	115	145	416	458	551	471	580	1,032	687	508
Mast Cells.....	12	11	29	14	9	11	0	0	15	17	0
Total Leucocytes..	6,116	5,500	7,266	6,820	4,720	5,300	4,533	5,800	7,700	8,580	7,940
Erythrocytes.....	9,500,000	10,600,000	8,420,000	8,740,000	8,240,000	8,240,000	7,600,000	8,480,000	8,680,000	8,000,000	8,920,000

Differential count after injection of hæmolytic serum (Sheep 9).

TABLE IV.

Time.	Nov. 6, 11 A.M. 3 hours before injection.	Nov. 6, 5 P.M. 3 hours after injection.	Nov. 6, 10.30 P.M.	Nov. 7, 9.30 A.M.	Nov. 7, 7.30 P.M.	Nov. 8, 10.30 A.M.	Nov. 9, 10.30 A.M.	Nov. 10, 9 A.M.
Lymphocytes.....	3,802	3,187	4,038	5,380	5,077	4,355	5,933	2,265
Large Mononuclears..	630	383	458	415	521	683	750	254
Amphophiles.....	3,353	4,845	8,102	6,100	4,804	2,791	4,046	4,741
Eosinophiles.....	515	68	51	305	423	404	614	0
Mast Cells.....	0	17	51	0	0	0	23	0
Total Leucocytes.....	8,300	8,500	12,700	12,200	10,825	8,233	11,366	7,260
Erythrocytes.....	8,428,000	9,216,000	4,824,000	7,000,000	6,800,000	8,560,000	8,780,000	9,360,000

Differential count after injection of hæmolytic serum (Sheep 3).

TABLE V.

Time.	Jan. 22, 10 A.M. 3 hrs. before injection.	Jan. 22, 5 P.M. 4 hrs. after injection.	Jan. 23, 1 A.M.	Jan. 23, 9 A.M.	Jan. 23, 9 P.M.	Jan. 24, 9 A.M.	Jan. 24, 9 P.M.	Jan. 25, 9 A.M.	Jan. 26, 9 A.M.	Jan. 27, 9 A.M.	Jan. 28, 9 A.M.	Jan. 29, 9 A.M.
Lymphocytes.....	4,996	4,581	6,317	5,450	5,628	6,344	6,552	6,194	5,155	4,932	5,946	5,747
Large Mononuclears.....	324	149	380	582	369	458	532	488	414	201	698	538
Amphophiles.....	3,032	3,916	4,330	4,243	4,300	3,739	2,879	3,841	2,732	3,311	3,471	2,127
Eosinophiles.....	154	96	111	125	243	359	449	566	319	307	444	111
Mast Cells.....	34	18	22	0	0	0	21	11	0	9	21	17
Total Leucocytes.....	8,540	8,760	11,160	10,400	10,540	10,900	10,433	11,100	8,620	8,760	10,580	8,540
Erythrocytes.....	13,400,000	16,000,000	15,200,000	15,760,000	13,400,000	12,920,000	12,840,000	13,880,000	13,780,000	15,120,000	13,200,000	13,680,000

Differential count after injection of hæmolympocytotoxic serum (Sheep 6).

TABLE VI.

Time.	Apr. 23, 10 A.M. Before injection.	Apr. 23, 4 P.M. 3 hrs. after injection.	Apr. 23, 9 P.M.	Apr. 24, 9 A.M.	Apr. 24, 9 P.M.	Apr. 25, 9 A.M.	Apr. 25, 9 P.M.	Apr. 26, 9 A.M.	Apr. 27, 9 A.M.	Apr. 28, 9 A.M.
Lymphocytes.....	6,054	2,888	2,660	7,072	5,711	4,722	5,039	5,082	4,541	5,141
Large Mononuclears.....	752	418	540	952	553	781	979	912	955	769
Amphophiles.....	2,350	5,342	6,680	5,358	3,419	3,450	4,080	4,692	7,275	4,089
Eosinophiles.....	188	35	60	109	138	54	41	109	0	20
Mast Cells.....	56	17	60	109	59	73	61	65	129	101
Total Leucocytes.....	9,400	8,700	10,000	13,600	9,880	9,080	10,200	10,860	12,900	10,120
Erythrocytes.....	4,210,000	4,800,000	4,300,000	3,810,000	3,930,000	3,600,000	3,240,000	3,550,000	3,600,000	3,850,000

Differential count after injection of hæmolympocytotoxic serum (Sheep 12).

TABLE VII.

Time.	Apr. 23, 11 A.M. Before injection.	Apr. 23, 5 P.M. 3 hrs. after injection.	Apr. 23, 10 P.M.	Apr. 24, 10 A.M.	Apr. 24, 10 P.M.	Apr. 25, 10 A.M.	Apr. 25, 10 P.M.	Apr. 26, 10 A.M.	Apr. 27, 10 A.M.	Apr. 28, 10 A.M.
Lymphocytes.....	3,077	2,424	3,444	3,394	3,120	3,178	2,775	2,385	2,863	2,506
Large Mononuclears.....	690	603	806	666	1,040	609	623	525	618	688
Amphophiles.....	3,105	2,955	3,571	4,226	3,760	2,820	3,962	4,515	3,158	3,910
Eosinophiles.....	0	12	32	17	32	0	15	15	27	14
Mast Cells.....	28	36	47	17	48	13	45	60	54	42
Total Leucocytes.....	6,900	6,030	7,900	8,320	8,000	6,620	7,420	7,500	6,720	7,160
Erythrocytes.....	5,100,000	4,820,000	5,070,000	5,200,000	5,340,000	4,280,000	4,350,000	4,600,000	5,080,000	5,180,000

Differential count after injection of hæmolytic serum (Sheep 14).

TABLE VIII.

Time.	Nov. 18, 11 A.M. Before injection.	Nov. 18, 7 P.M. 4 hrs. after injection.	Nov. 18, 12 P.M.	Nov. 19, 9 A.M.	Nov. 19, 8 P.M.	Nov. 20, 9 A.M.	Nov. 20, 4 P.M.	Nov. 21, 9 A.M.	Nov. 22, 9 A.M.	Nov. 23, 9 A.M.
Lymphocytes.....	3,953	3,200	3,827	5,148	3,486	3,977	6,595	5,693	3,320	5,606
Large Mononuclears.....	533	508	373	616	458	512	567	513	318	283
Amphophiles.....	2,326	4,458	4,531	5,461	3,856	4,959	7,296	2,781	3,320	6,258
Eosinophiles.....	374	167	169	372	233	372	432	308	275	148
Mast Cells.....	14	0	0	23	0	0	30	38	0	25
Total Leucocytes.....	7,200	8,333	8,900	11,620	8,033	9,820	14,920	9,333	7,233	12,320
Erythrocytes.....	9,600,000	8,540,000	9,560,000	9,220,000	9,640,000	9,460,000	9,440,000	9,630,000	9,300,000	8,860,000

Differential count after injection of heated hæmolytotoxic serum (Sheep 4).

TABLE IX.

Time.	May 16, 10 A.M. Before injection.	May 16, 4 P.M. 3 hrs. after injection.	May 16, 9 P.M.	May 17, 9 A.M.	May 17, 9 P.M.	May 18, 9 A.M.	May 18, 9 P.M.	May 19, 9 A.M.	May 20, 9 A.M.	May 21, 9 A.M.
Lymphocytes.....	6,484	5,312	6,739	7,680	8,660	7,962	7,530	6,733	6,752	5,984
Large Mononuclears.....	1,138	864	1,152	1,220	1,205	1,076	1,511	1,210	1,060	1,091
Amphophiles.....	1,687	1,728	1,498	1,262	893	1,162	1,906	1,814	1,153	1,408
Eosinophiles.....	38	48	153	316	380	517	604	262	260	246
Mast Cells.....	133	48	58	42	22	43	69	61	75	71
Total Leucocytes.....	9,480	8,000	9,600	10,520	11,160	10,760	11,620	10,080	9,300	8,800
Erythrocytes.....	12,290,000	12,950,000	10,800,000	11,460,000	11,890,000	12,180,000	11,940,000	12,400,000	12,020,000	12,000,000

Differential count after injection of heated haemolymphotoxigenic serum (Sheep 19).

TABLE X.

Time.	Apr. 23, 9 A.M. Before injection.	Apr. 23, 3 P.M. 3 hrs. after injection.	Apr. 23, 8 P.M.	Apr. 24, 8 A.M.	Apr. 24, 8 P.M.	Apr. 25, 8 A.M.	Apr. 25, 8 P.M.	Apr. 26, 8 A.M.	Apr. 27, 8 A.M.	Apr. 28, 8 A.M.
Lymphocytes.....	4,485	2,033	1,075	1,776	5,433	3,126	3,080	2,096	3,028	4,037
Large Mononuclears.....	607	455	202	365	664	563	940	629	985	765
Amphophiles.....	1,573	2,275	4,233	6,026	3,863	3,126	3,403	2,347	1,775	1,949
Eosinophiles.....	138	48	56	83	60	141	46	84	110	42
Mast Cells.....	97	29	34	58	40	84	231	84	182	167
Total Leucocytes.....	6,900	4,840	5,600	8,300	10,060	7,040	7,700	5,240	6,080	6,960
Erythrocytes.....	6,670,000	7,740,000	4,420,000	6,400,000	5,200,000	6,060,000	5,890,000	6,050,000	5,760,000	6,260,000

Differential count after injection into a rabbit of haemolymphotoxigenic serum prepared for sheep.

TABLE XI.

Time.	Apr. 9, 11 A.M. Before injection.	Apr. 9, 4.30 P.M. 3 hrs. after injection.	Apr. 9, 9.30 P.M.	Apr. 10, 9.30 A.M.	Apr. 10, 9.30 P.M.	Apr. 11, 9.30 A.M.	Apr. 11, 9.30 P.M.	Apr. 12, 9.30 A.M.	Apr. 13, 9.30 A.M.	Apr. 14, 9.30 A.M.	Apr. 15, 9.30 A.M.
Lymphocytes.....	4,563	2,997	3,018	4,798	5,219	4,144	4,128	4,805	5,643	4,569	3,965
Large Mononuclears.	607	349	338	683	618	602	564	574	756	558	520
Amphophiles.....	2,365	3,101	3,960	2,832	2,004	2,184	2,064	2,201	2,785	3,488	1,976
Eosinophiles.....	45	13	15	17	16	0	110	62	18	35	13
Mast Cells.....	0	0	29	0	63	70	14	108	18	70	26
Total Leucocytes..	7,580	6,460	7,360	8,330	7,920	7,000	6,880	7,750	9,220	8,720	6,500
Erythrocytes.....	5,160,000	5,020,000	4,320,000	4,000,000	3,620,000	3,360,000	3,280,000	3,800,000	3,620,000	3,040,000	2,760,000

Differential count after injection of endothelotoxic serum (Sheep 15).

TABLE XII.

Time.	Apr. 5, 10 A.M. Before injection.	Apr. 5, 4.30 P.M. 3 1/2 hrs. after injection.	Apr. 5, 10.30 P.M.	Apr. 6, 9 A.M.	Apr. 6, 9 P.M.	Apr. 7, 9 A.M.	Apr. 7, 9 P.M.	Apr. 8, 9 A.M.	Apr. 9, 9 A.M.	Apr. 10, 9 A.M.	Apr. 11, 9 A.M.
Lymphocytes.....	3,147	1,399	1,616	2,435	3,304	3,103	3,454	2,761	3,780	3,786	4,336
Large Mononuclears.	543	318	231	423	552	377	490	490	378	689	671
Amphophiles.....	1,706	4,116	9,952	7,122	5,644	3,511	2,679	1,931	2,772	2,204	3,059
Eosinophiles.....	111	12	23	60	294	145	163	138	70	67	98
Mast Cells.....	33	35	23	20	39	14	14	0	0	14	16
Total Leucocytes..	5,540	5,880	11,545	10,060	9,833	7,240	6,800	5,320	7,000	6,760	8,180
Erythrocytes.....	6,420,000	7,440,000	7,020,000	6,800,000	5,500,000	5,800,000	6,060,000	6,020,000	5,620,000	4,920,000	4,000,000

Differential count after injection of hepatotoxic serum (Sheep 16).

TABLE XIII.

Time.	Apr. 14, 11 A.M. Before injection.	Apr. 14, 4 P.M. 3 hrs. after injection.	Apr. 14, 9 P.M.	Apr. 15, 9 A.M.	Apr. 15, 9 P.M.	Apr. 16, 9 A.M.	Apr. 16, 9 P.M.	Apr. 17, 9 A.M.	Apr. 18, 9 A.M.	Apr. 19, 9 A.M.	Apr. 20, 9 A.M.	Apr. 21, 9 A.M.
Lymphocytes	2,866	1,843	1,995	2,762	3,771	3,480	3,496	3,414	2,451	3,443	3,288	4,730
Large Mononuclears	461	186	367	510	505	696	654	553	548	653	499	836
Amphophiles	1,883	5,431	3,746	2,343	2,311	1,812	1,736	1,883	3,197	3,294	7,484	5,390
Eosinophiles	10	0	12	34	53	12	48	24	12	15	23	22
Mast Cells	10	0	0	11	0	0	12	12	12	15	46	22
Total Leucocytes	5,230	7,460	6,120	5,660	6,640	6,000	5,946	5,886	6,220	7,420	11,340	11,000
Erythrocytes	7,180,000	6,920,000	7,300,000	7,500,000	7,820,000	7,320,000	6,580,000	6,780,000	6,800,000	7,200,000	6,800,000	7,000,000

Differential count after injection of splenotoxic serum (Sheep 18).

TABLE XIV.

Time.	Apr. 1, P.M. Before injection.	Apr. 1, 5 P.M. 3 hrs. after injection.	Apr. 1, 12 P.M.	Apr. 2, 9.30 A.M.	Apr. 2, 6.30 P.M.	Apr. 3, 9.30 A.M.	Apr. 3, 7.30 P.M.	Apr. 4, 8.30 A.M.	Apr. 5, 8.30 A.M.	Apr. 6, 8.30 A.M.	Apr. 7, 8.30 A.M.
Lymphocytes	4,180	3,270	2,800	5,581	5,183	5,532	3,487	4,923	3,739	5,460	5,163
Large Mononuclears	562	413	552	1,243	860	734	716	926	566	575	740
Amphophiles	3,303	3,881	2,751	3,673	2,083	1,714	2,885	2,313	3,025	2,873	2,269
Eosinophiles	198	46	37	381	99	131	36	49	15	36	32
Mast Cells	17	30	0	22	41	49	36	49	15	36	16
Total Leucocytes	8,260	7,640	6,140	10,900	8,266	8,160	7,160	8,260	7,360	8,980	8,220
Erythrocytes	9,720,000	10,100,000	9,200,000	9,640,000	8,820,000	9,000,000	9,000,000	9,200,000	9,320,000	9,000,000	8,800,000

Differential count after injection of nephrotoxic serum (Sheep 17).

TABLE XV.

Time.	Mar. 25, P.M. Before injection.	Mar. 25, 5.30 P.M. 3 hrs. after injection.	Mar. 25, 10.30 P.M.	Mar. 26, 8.30 A.M.	Mar. 26, 8.30 P.M.	Mar. 27, 8.30 A.M.	Mar. 27, 8.30 P.M.	Mar. 28, 8.30 A.M.	Mar. 29, 8.30 A.M.	Mar. 30, 8.30 A.M.	Mar. 31, 8.30 A.M.
Lymphocytes.....	2,857	1,491	1,470	2,300	2,699	2,491	2,242	3,067	2,767	4,235	2,965
Large Mononuclears.	379	136	124	289	305	202	235	270	262	494	349
Amphophiles.....	2,970	2,616	4,542	5,427	3,681	2,586	4,387	3,720	4,421	5,468	3,894
Eosinophiles.....	101	17	44	24	221	21	56	43	30	62	43
Mast Cells.....	13	0	0	0	14	0	0	0	0	21	15
Total Leucocytes..	6,320	4,260	6,180	8,040	6,920	5,300	6,920	7,100	7,480	10,280	7,266
Erythrocytes.....	4,426,000	5,080,000	5,240,000	5,660,000	4,960,000	5,740,000	4,960,000	4,960,000	5,120,000	4,980,000	4,580,000

Differential count after injection of lymphotoxic serum (Sheep 11).

MWCLIFT
SEC

FIG. 1.

MWCLIFT
SEC

FIG. 2.

FIG. 3.

FIG. 4.