Leucocytosis, and the Effect of Drugs on the Leucocytes of the Intravascular Blood.

Thesis for degree
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Part I Leucocytosis

Part II The effect of drugs on the Leucocytes of the Intravascular Blood.

Part I

Leucocytosis

Definition. The term Leucocytosis was first used by Virchow to designate a change in the blood which consists in a moderate increase in the number of the white corpuscles, & which accompanies certain pathological conditions. It is distinguished from the change in the blood found in the disease Leukæmia by the fact that the increase in the number of leucocytes in leucocytosis is much less in degree, & usually of shorter duration: as well as by the presence in leukæmia, of changes in the lymphatic glands & other tissues, which are ~~also~~ always present in the latter disease.

It has been suggested (Tagge's Principles & Practice of Medicine 2nd edition vol II p. 753) that when the proportion of white cells to red, in the blood, reaches 1 to 20, the condition is one of leukæmia: when the proportion of white is less than this, but still distinctly above the normal, the condition is one of leucocytosis.

The blood of leukæmia may be further distinguished from that of simple leucocytosis, by the fact that the relative proportion of the different varieties of leucocytes present, is usually markedly different in the two conditions.

Perhaps the best definition of Leucocytosis, is

that of any increase in the number of leucocytes in the blood, which is not Leukæmia.

Besides the pathological conditions in which Leucocytosis is present, there are certain normal physiological states in which the number of leucocytes in the blood is temporarily much increased. These may be described as conditions of Physiological Leucocytosis, to distinguish them from ~~these~~ the leucocytosis of pathological conditions. The latter may be termed Pathological Leucocytosis.

Before entering into more details regarding leucocytosis it will be well to describe shortly the leucocytes of normal human blood.

Number of leucocytes. The number is variously stated by different authors, but may be said to average 6000 to 10,000 per cubic millimetre: the proportion of white cells to red being from 1:500 to 1:800

Classification of leucocytes. They may be divided into the following 5 varieties:-

I Small cells with a single spherical nucleus, surrounded by a very narrow rim of protoplasm. They are not larger than the red corpuscles. The nucleus stains deeply, & the protoplasm is free from granules. They are often called lymphocytes.

II Larger cells, also round, with more protoplasm. The single nucleus is usually spherical but sometimes kidney-shaped, & stains less deeply than that of the first-variety. The protoplasm contains no definite granules.

III Cells usually larger than red corpuscles, with a nucleus of very irregular form, & protoplasm containing a large number of very fine granules. The granules, according to Ehrlich's classification, are neutrophile, but Kanthack & Hardy (Journal of Physiology Vol XVII p. 84) & some other later observers, regard them as really oxyphile in reaction. These cells are the most numerous of all the varieties, being estimated at 70 per cent: or more of the total number of leucocytes.

IV Cells larger in size than any of the others, with an irregular nucleus, & protoplasm which is characterised by the presence of relatively large, highly refractive granules, which stain deeply with eosin & other acid dyes. They are the "eosinophile cells" of Ehrlich. They are present in small numbers only, forming 2-4 per cent: of the total number of leucocytes.

V A group of small cells, with irregular nuclei, & protoplasm which contains a large number of fine granules which stain readily with methylene blue. They are present in small numbers - 1 to 5 per cent: They are the basophile cells with 8 granulations of Ehrlich.

The Leucocyte as a Cell.

It will be convenient to give here a very brief summary of some points connected with the constitution & supposed functions of the leucocyte. First, however, with regard to the relation of the different varieties to each other. It appears to be generally accepted at the present time that the small mononuclear cell or "lymphocyte" is formed in lymphatic glands & lymphoid tissues generally: and further, that the lymphocyte is the youngest form of the leucocyte. There seems to be little doubt also, that the large mononucleated hyaline cell is developed directly from the lymphocyte; their general resemblance, & especially the fact that every transitional form between the two can be found, naturally suggests this. With regard to the origin of the granular cells with irregular nuclei, and their relation to the lymphocytes, there is more difference of opinion. Metschnikoff ("Lect. on Inflammation" translated by Starling, p. 114) states that the ordinary polynuclear cells develop in the blood from the small cells, & he quotes the work of Ruskoff in support of this statement. The large coarsely-granular eosinophile cells were said by Ehrlich to be produced especially in the bone-marrow. Gulland (Roy. Soc. Proc. Vol LIX p. 73) states that they are simply the finely granular cells which have grown larger in size, while the contained granules have become more strongly oxyphile as well as larger. If Gulland's view is the correct one, why are the coarsely granular cells so

definitely separated from the finely granular groups? One would expect to find every transition between the two groups, but transitional forms between them appear to be very exceptional, if they exist at all. Sherrington expresses doubts about Ehrlich's view of the origin of these cells from the oxyphile marrow-cells (Roy. Soc. Proc. Vol. LV. p. 204). The basophile cells according to Gulland (loc. cit.) are derived from the hyaline; but little appears to be definitely known about this group of leucocytes.

Nature & Significance of the Granules in the Protoplasm. The granules, like the rest of the protoplasm, are albuminous or proteid in character. The difference in their staining reactions to acid, alkaline, & neutral dyes, would seem to indicate a corresponding difference in the reaction of the granules themselves. Halliburton (Goulst. Lect. on "The Chemical Physiology of the Animal Cell", Brit. Med. Jour. Vol. I 1893, p. 506) suggests that the presence of these granules is possibly a proof of the changing chemical reaction of the cell-contents at different periods of its assimilative activity. The reaction of living protoplasm as a whole is alkaline but various acid products are formed as the result of the protoplasmic activity of cells, e.g. CO_2, lactic & uric acids &c: and the acid reaction becomes permanent when the cell is dying from want of new material to correct the katabolic changes, which become predominant & result in the formation of lactic acid & acid phosphates.

The instability of the chemical reaction is also shown by an experiment of Kanthack & Hardy, who found that eosinophile cells, after feeding on bacteria, became amphophile. (Roy. Soc. Proc. LII. p. 27).

In this connection it may be mentioned that Dr Buchanan of Liverpool, has discovered in leukæmic blood, a cell which contains both oxyphile & basophile granules. (Brit. Med. Jour. – Report of meeting of Liverpool Med. Inst. – April 18th 1896. – p. 978)

The metabolic activity of the leucocyte is a matter of great importance in considering the function of the cell, and has not yet been sufficiently worked out.

Is the coarsely granular leucocyte a secreting gland? This idea has been brought forward by Ehrlich & others: and there is apparently some evidence in its favour. Hankin has arrived at the conclusion that the alexenes or protective proteids which confer bacteria-killing power on blood-serum, originate from the coarsely-granular eosinophile cells: and the shedding of fibrin-ferment which causes the coagulation of blood, appears to take place largely from these cells. The shedding of the ferment is accompanied by a loss of the granules, or a change in their position relative to the rest of the protoplasm (Wallibarton – Lect. – loc. cit. p. 631) This suggests a comparison between the process & true secretion. Kanthack & Hardy (Roy. Soc. Proc. Dec. 1892) have proved that under bacterial irritation, the coarsely granular leucocyte of the frog suffers a more or less

complete loss of its granules, while the surrounding microbes suffer damage.

Amœboid & phagocytic activity of leucocytes.

Whether all varieties of leucocyte are capable of amœboid activity is not certain, but they undoubtedly possess it in different degrees. Most authorities deny that the lymphocytes are amœboid, but Metschnikoff (loc. cit. p. 115) states that they are. All agree that the polynuclear "neutrophiles" are actively amœboid: & according to Metschnikoff, the large mononuclear are equally so. Sherrington states that the latter are sluggishly amœboid. The coarsely granular cells is less amœboid than the finely granular (Sherrington) The power of englobing foreign particles (phagocytosis) is possessed in a high degree by the large mononuclear, & polynuclear "neutrophiles" (Metschnikoff): the lymphocytes are devoid of this power: & the large eosinophiles are also non-phagocytic (Metschnikoff: & Sherrington). This note of some points connected with the leucocyte is given here on account of the relation of these to the theories which have been advanced in explanation of the phenomena of leucocytosis.

Physiological Leucocytosis

occurs under the following conditions.

I In the newly-born child. During the first 2 or 3 days, the number of leucocytes is at least double that in the adult. Messrs Elder & Hutchinson in some recent observations (Edin. Obstet. Trans. Vol XX 1894-5) state that in 12 cases in which they examined the blood at birth, the average number was 17, 884 per cubic millimeter. Hayem & other previous observers have given considerably higher figures. The increase appears to be principally due to the relatively large proportion of small mononuclear leucocytes.

II During pregnancy. In 11 cases examined by Elder & Hutchinson, the average per c. mm. was 14, 522

III During digestion. The occurrence of leucocytosis during the digestive process has long been known. One or two hours after the principal meal, leucocytes are found in the blood in the proportion of 1 to 150, or even 1 to 100 of the red corpuscles (v. Jaksch, Clin. Diagnosis p. 28) Soon afterwards the proportion becomes normal again. The variety of leucocyte which is specially increased is the small mono. nuclear lymphocyte. Kanthack & Hardy (loc. cit. p. 98) state that two hours after a full meal, the lymphocytes may form as many as 30 per cent. of the total number of white corpuscles. The finely granular oxyphile

+ Cagney:

cells are relatively diminished. Kanthack & Hardy also state that the finely granular basophile cells are increased in number & granularity shortly after a full meal; the granulation becoming less numerous & distinct after the digestive process is completed.

Pathological Leucocytosis

Virchow, long ago, stated that there is an increase in the number of leucocytes in the blood, in all conditions in which the lymphatic glands are irritated. More recent observations have proved that an increase is associated with a great variety of morbid conditions. Von Limbeck states that it always accompanies inflammatory processes in which exudation occurs, & he proposes to give this form the name of "inflammatory leucocytosis". He says, for example, that it occurs in croupous pneumonia & suppurative peritonitis, but not in the infectious diseases which give rise to no exudation into the tissues, e.g. typhoid, intermittent fever, septic fever, even if the fever rises very high

Diseases which are accompanied by Leucocytosis

Anæmia, due to loss of blood. Osler (Principles & Practice of Medicine 2nd edition 1895) states that there is a moderate leucocytosis, a differential count showing an increase of the multinuclear neutrophiles, with relative diminution of the small mononuclear elements.

Chlorosis. Osler states that there is often a slight leucocytosis. Its occurrence is also mentioned by v. Jaksch (Clin. Diagnosis)

Pernicious Anæmia. v. Jaksch states that there is a leucocytosis; but Osler says the leucocytes are generally normal or diminished in number, while in the graver cases, a marked increase in the small mononuclear cells with diminution of the polynuclear leucocytes is often noted.

Sarcoma & Carcinoma. In the Traité de Médecine of Charcot, Bouchard & Brissaud, 1892 vol II p.485, it is stated that 10,000 to 20,000 leucocytes per c.mm. are frequently counted in these affections: and M. Hayem is stated to have found 50,000 in a case of sarcoma of bone, & 70,000 in a case of cancer of the thyroid body. Osler states that in a case of cancer of the stomach Welch found the proportion of white cells to red as high as 1 to 20.

Acute Rheumatism. Leucocytosis well-marked (Osler: also Charcot, Bouchard & Brissaud)

Acute Gout. Leucocytosis present (Charcot, Bouchard & Brissaud) loc. cit.

In Pleurisy + Empyema. In the latter especially the leucocytosis is marked (Osler)

Pyæmia. Leucocytosis marked (Osler). Also present in Cerebro-spinal Meningitis (Osler). And in Tubercular Meningitis (Charcot Bouchard + Brissaud)

In Bronchitis + "La Grippe" (" " ")

In Acute Tuberculosis Osler states that leucocytosis is slight or absent. Whilst in Chronic Pulmonary Tuberculosis (Phthisis) there is a marked increase in the number of leucocytes, especially in the later stages (Osler).

Ehrlich has shewn that the mononuclear leucocytes are markedly increased in the blood of phthisical patients. (Metchnikoff - Lect: on Compar. Pathology of Inflammation, translation by Starling p. 168 ~~121~~.)

Pneumonia. The leucocytosis which usually accompanies this disease has been studied by many observers. It appears early, and it is stated that the number of leucocytes varies as the temperature varies: when the disease terminates in crisis, the number of leucocytes decreases suddenly, whilst in cases where the temperature falls slowly (lysis), the fall in the number of leucocytes is also gradual. Osler (op. cit. p. 534) states that the number of leucocytes per c.mm. may rise to 40,000 or 50,000 or even more. In a discussion on croupous pneumonia reported in the British Med. Journal for Nov. 9th 1895, Dr Douglas Powell mentioned amongst other cases, one in which the

proportion of white cells to red, was 1 to 100: & Dr. G. A. Gibson stated that in one of his cases the proportion was 1 to 70. In "malignant pneumonia" the leucocytosis is absent (Osler)

In croupous pneumonia of children, v. Jaksch states that he has never failed to find it (Clin. Diagnosis)

Scarlet-Fever. Osler (op. cit. p. 78) states that there is a leucocytosis in this disease: but Dr. G. A. Gibson in the course of the above-mentioned discussion, asserted that it is not present.

Erysipelas. Leucocytosis is here a constant phenomenon. It is at its height during the febrile period, when the blood contains a number of living streptococci, & comes to an end after the crisis, when only masses of dead microbes are to be found in the organism (v. Limbeck & Pèr, quoted in Metchnikoff's Lectures p. 121)

Diphtheria. Numerous observations in this disease have been recently recorded: especially those of Gabritschewsky (Ann. del' Institut Pasteur 1894), & J. L. Morse (Boston Med. & Surg. Journal Mar. 7, 1895) may be mentioned. Morse observed 30 cases: in all there was a decided leucocytosis, well-marked by the 3rd day, & in a general way increasing during the progress of the disease until the latter had reached its height: diminishing during convalescence, & disappearing with, or soon after, the membrane. He considers the leucocytosis to be due to the absorption of toxines from the fauces.

In the majority of cases, the increase was chiefly in the polynuclear neutrophiles.

Febrile Diseases not accompanied by Leucocytosis

Measles according to Osler (op. cit. p. 84)

Typhoid Fever. Osler (op. cit. p. 18) as the result of observations made by W.S. Thayer, states that the number of leucocytes varies little from the normal standard. They diminish slightly during the course of the fever & reach the lowest point towards the end of convalescence. Sadler (quoted by v. Jaksch Clin. Diagnosis) says that when leucocytosis occurs in the course of this fever, it is due to the occurrence of some suppurative disorder as a complication.

Malaria. J. S. Billings (Johns Hopkins ~~Hosp~~ Hosp. Bulletin 1894 No 42) has investigated the change in the number of leucocytes in the blood. In certain ague, he finds a striking diminution during the febrile stage. This progressively continues until the minimum is reached at the end of a paroxysm, when the temperature is subnormal. The number then rises somewhat, & during the interval occupies a position midway between the maximum & minimum. The large mononuclear cells are as a rule greatly increased, both absolutely & relatively

Osler (op. cit. p. 171) confirms this statement: & further says that in typhoid also, there is a relative increase in the large mononuclear cells, with relative diminution of the polynuclear.

Diagnostic value of Leucocytosis in disease.

As will be gathered from the preceding statements, the occurrence or absence of leucocytosis in any particular case, may prove of some diagnostic importance. If Osler's statements of the usual occurrence of leucocytosis in scarlet-fever, & its absence in measle, are correct, ~~it may~~ the counting of the white blood-corpuscles may assist in the diagnosis of a doubtful case. More important assistance may be gained in the diagnosis of a suspected case of typhoid fever from some of the diseases which are apt to be mistaken for it, or vice versa.

Hartung (Wein. Med. Woch. Oct 3rd & 10th 1895: quoted in Brit. Med. Journal Epitome. No 577 Nov. 9. 95) has suggested an ingenious method of diagnosis of cancer of the stomach from simple ulcer. Schreyer discovered that the marked leucocytosis which follows an albuminous meal in a healthy subject, did not occur in patients suffering from cancer of the stomach. Hartung confirmed this observation in 10 cases of cancer in which he tried the effect of a meal of albuminous food – eggs, milk etc to which 1 grain of nuclein was added. This meal caused no increase in the leucocytes of the cancer patients while in healthy subjects it caused an increase amounting sometimes to as much as 96 per cent: in 3½ hours. In all the cancer cases he found a diminution of the red cells with increase of the white, particularly of the

polynuclear forms: & in most the eosinophile cells were diminished.

Blinderman's (Wien. med. Blätt. Oct. 31. 95 – in Brit. med. Journal Epitome Dec. 14. 95) records some observations which confirm those of other workers, and concludes that the increase in the number of leucocytes, with the marked diminution in the red cells & in the haemoglobin, which occurs in cases of cancer of the stomach, always affords a means of diagnosis between this & other gastric disorders.

Relation of Leucocytosis in Disease, to Prognosis & Treatment

In two diseases – pneumonia & diphtheria – some investigations have been made as to the bearing of leucocytosis on prognosis.

In pneumonia, v. Jaksch (Clin. Diagnosis), Osler (op. cit.) & J. A. Gibson (Brit. med. Journal Nov. 9. 95) state that the prognosis is bad when leucocytosis is only slight, or absent. This statement is probably partly based on the theoretical consideration that the leucocytes act as phagocytes & thus assist the patient in his struggle against the specific microbe: hence, the more leucocytes the better for the patient. v. Jaksch (Centralbl. f. klin. med. Feb. 6. 1892) recommends that in cases in which the leucocytosis is deficient, drugs which cause an increase in the number of leucocytes, should be given: and he reports a case in which the number was

increased by giving pilocarpin. Probably few physicians would agree with him in the use of such a remedy, but in the present state of our knowledge would leave the leucocytosis out of consideration, & rely principally on the free use of stimulants – and probably to the greater advantage of the patient.

The real significance & importance of leucocytosis in this & other diseases, is by no means certain.

Haegele-Akerblom (Brit. med Journal Epit. Aug. 24. 95) attributes the good results of treatment of pneumonia by digitalis, partly to the increase of leucocytes which his investigations in animals & man show is produced by it.

In diphtheria, Gabritschewsky (Ann. del'Inst. Past. 1894) as the result of the examination of a number of cases, concludes that the progressive increase in the number of leucocytes gives a bad prognosis, as it indicates a greater degree of infection of the organism by the diphtheritic poison: although at the same time the increased leucocytosis is valuable in combating the infection (by phagocytic action)

Experimental Investigations in Leucocytosis

During recent years numerous investigations have been made, & the results published, some of the more important of which will be given in more detail below. However, it may be stated here that one important result has been arrived at, viz., that the increase in the number of leucocytes to which the name of leucocytosis was at first limited, is preceded, when the condition is induced experimentally, by a preliminary stage in which the number of leucocytes in the circulating blood falls below the normal number.

Hence the phenomena of leucocytosis may be divided into 2 stages, each of which will be separately considered.

(1) The first stage, consisting in a diminution of the number of leucocytes in the circulating blood, usually lasts only a comparatively short time, & then merges into the second. Various observers have distinguished it under different names. Löwit, who has devoted special attention to it, calls it (on theoretical grounds to be afterwards explained) leucolysis. For the diminution which, he suggests, will be found to precede the increase in all cases of inflammatory leucocytosis, he has introduced the term leucocytopenia. The latter has been adopted by C. S. Sherrington (Proc. Roy. Soc. 1894 No 331). French workers generally use the term hypoleucocytosis.

(2) The second stage is generally of much longer duration, & more marked in degree. It consists in an increase in the number of leucocytes in the circulating blood, and is essentially the leucocytosis of those observers who first noted the condition, many of whom overlooked the preceding diminution, owing to lack of sufficiently early examination of the blood after the experiment. French observers usually distinguish it under the name of hyperleucocytosis

The following is a short summary of some of the more recent papers on the experimental production of leucocytosis, each of the stages being considered separately for convenience, although there is not actually any sharp line of demarcation between them.

I Leucocytopenia or Hypoleucocytosis

Wernigo (Les Globules Blancs comme Protecteurs du Sang: Ann. de l'Inst. Pasteur (892, p.478) injected into the veins of rabbits, cultures of Bacillus prodigiosus (a non-pathogenic organism) both in the living state, & also after killing the organisms by exposing the culture to a high temperature: also, cultures of Bacillus pyocyaneus, (both living & dead); of bacteria of Hog-Cholera; of bacillus tuberculosis; and of B. anthracis. With each of these, he succeeded in producing a diminution in the number of circulating leucocytes,

which was followed by a more or less marked increase (hyperleucocytosis).
After the injection of a culture of B. pyocyaneus, previously filtered by means of Chamberland's filter, he did not observe any diminution, but instead, an increase in the number of circulating leucocytes, which was well-marked half an hour after the injection. In another experiment, he injected a quantity of powdered carmine mixed with water, directly into the circulation: this was followed by ~~hy~~hypoleucocytosis like the bacterial injections, but differing in the number of leucocytes returning to normal without any hyperleucocytosis.

Römer (Die chemische Reizbarkeit thierischer Zellen: Virchow's Archiv. 1892.) working also with rabbits, caused a diminution by the injection of dead cultures of the potato-bacillus, and by the injection (both intravenous + subcutaneous) of filtered cultures of the same organism. Also, by the injection of extracts of bacterial cultures.

Kanthack + Hankin (Proc. Cambridge Phil. Soc. Jan 1892) have observed an analogous reaction after the injection of sterilised cultures into the ear-veins of rabbits.

Kanthack (Brit. med. Journal 1892 Vol. I p. 1301: Acute leucocytosis produced by bacterial products) states that the injection into the ear-vein of rabbits, of filtered cultures of B. pyocyaneus, & of yeast, ~~gave~~ causes a diminution followed by an increase, just as after the injection of B. Metschnikovi

A. E. Wright (Proc. Royal Soc. Feb. 9. 93) injected 10 per cent. solution (in .75 per cent. solution of NaCl) of peptone, in dogs & rabbits. This was followed by an enormous diminution in the circulating leucocytes, more marked in the dogs than in the rabbits. In one of the former animals, the number fell from 15.900 per c.mm. before the experiment, to 400 ten minutes after.

Everard, Demoor & Massart (Sur les modifications des Leucocytes dans l'Infection et dans l'Immunisation: Ann. de l'Institut-Pasteur Feb. 1893) performed a series of experiments on rabbits & guinea-pigs, using for the purpose of injection, cultures of the Vibrio of metchnikoff, the bacillus of hog-cholera, the staphylococcus pyocyaneus aureus, the bacillus anthracis, the bacillus of tetanus, & the bacillus mycoïdes. With the V. metchnikoff, in addition to the virulent-living cultures, injections of cultures killed by heating to 100°C, & cultures filtered but-not-killed, were made. Besides the experiments on unprotected animals, a series of injections of the same microbes were made in animals which had been previously immunised against the respective bacteria. As the result of their observations, these authors state that the injection of microbic cultures, whether living or dead, causes first a diminution in the number of circulating leucocytes. With virulent-injections, death frequently occurs during this stage, but if the animal survives, the diminution is

followed by hyperleucocytosis. A similar result followed the injection of filtered cultures, in this respect their observations agreeing with those of Römer and Kanthack, and differing from those of Wenigo, as already stated. In ~~both~~ the protected animals (whose blood was found to be abnormally rich in leucocytes) the hypoleucocytosis was found to be less marked than in the unprotected.

P. F. Müller (Inaugural-Dissertation: Berlin 1894) injected cultures of B. pyocyaneus, diplococcus pneumonicus, potato-bacilli, streptococci, & bacilli of putrefaction. The animals (rabbits) were first anæsthetised with ether, & the right-jugular & femoral veins exposed: the cultures were injected into the former, & blood was taken from the latter for examination at short intervals after the injection. The diminution in the number of leucocytes in the femoral blood after the injection was very marked, & was noticeable in a surprisingly short space of time. In one experiment the number fell from 7200 per cmm. before the injection, to 6400, 55 seconds after it: and in most it was well marked within 4 or 5 minutes. After injecting streptococcus bouillon into an animal whose leucocytes were 10400 per cmm. before the experiment, the number fell in 2 minutes 50 seconds, to 1200 per c.mm. In these experiments, probably much of the diminution was caused by the operative procedures preliminary to the injections, since

Löwit (quoted by Sherrington; Proc. Roy. Soc. Vol XV) states that binding or tying down an animal is sufficient to produce severe changes in the blood, one of these changes being a diminution in the number of leucocytes.

C. S. Sherrington (Note on some changes in the blood of the general circulation consequent upon certain inflammations of acute & local character: Proc. Roy. Soc. Vol. XV. 1894) performed a series of experiments of an entirely different character from those previously mentioned. He used dogs & cats: and, avoiding bacterial & chemical irritants, he induced acute local inflammation in the limbs by immersing them in hot water, and (in other cases) by the application of hot sponges, & occasionally by a ligature, to a knuckle of bowel brought to a small incision in the linea alba; the operations being performed with strict precautions for asepsis. These procedures were in all cases followed by a slight diminution in the number of circulating leucocytes, this again being succeeded by a marked increase: in some cases there was noted much later, a fall again to below the normal number. Along with these changes an increase in the specific gravity of the blood was observed.

Relative proportion of the different varieties of circulating leucocytes during the leucocytopenic stage.

During the leucocytopenia consequent on on binding down an animal, prolonged exposure, cooling etc, Löwit (quoted by Sherrington, loc. cit.) states that the decrease is especially due to decrease in the mononuclear variety. On the other hand, during the diminution in the total number of leucocytes following the injection of bacteria, bacterial extracts, peptones & other foreign substances, all observers appear to agree that the polynuclear are those principally diminished, the mononuclear being relatively increased. Sherrington has shown that the same change occurs in the leucocytopenia caused by acute local inflammations.

Changes in the Organs during this stage: and Theory of Leucocytopenia.

Löwit who has specially studied this stage, found that many substances besides bacteria & their products, when injected into the veins of rabbits, cause a marked diminution of the circulating leucocytes. These were albumose, peptone, pepsin, nucleic acid, nuclein, leech extract, pyocyanin, tuberculin, curare, urea, uric acid, & sodium urate. He believes that these substances cause a destruction & dissolution of the leucocytes in the blood-plasma: hence he terms this stage "Leucolysis".

A. E. Wright (Proc. Roy. Soc. Feb. 9. 93.) has also experimented with peptone injections (10% solutions) on dogs & rabbits. His observations confirm those of Löwit as regards the consequent diminution in the number of leucocytes: and he comes to the same conclusion, that the leucocytes which have disappeared are dissolved in the plasma. He bases this opinion on a comparative examination of the blood of a mesenteric vein & the carotid blood; and on a histological examination of the liver, kidney & heart-muscle for leucocytes. He found no trace of stasis or of emigration of leucocytes in either dog or rabbit, after the peptone injection. This opinion, he believes is supported by the chemical properties of the peptone plasma, especially by the fact that it deposits on cooling a heavy precipitate of a nucleo-albumin, which he states is probably identical with Wooldridge's tissue-fibrinogen, i.e. with the characteristic albuminous constituent of white blood corpuscles. Further, the action of CO_2 on peptone plasma is explicable on the theory that this plasma contains disintegrated leucocytes in solution.

Halliburton & Brodie in the course of a research on the coagulation of blood, performed some experiments (Journal of Physiology Vol XVII, p. 172) which bear upon the theory of the destruction of leucocytes.

They found that solution of thymus nucleo-albumin

when injected into rabbits caused a marked diminution in the circulating leucocytes: a similar result ~~also~~ followed the injection of 5% solution of peptone: also 2% solution of Sodium Carbonate: 1% solution of $CaCl_2$ caused a diminution of both white and red corpuscles. They further observed the action of these substances on drops of blood withdrawn from the circulation, on a microscopic slide. The nucleo-albumin at once caused the leucocytes to break up into tiny granules. The peptone had no effect on the leucocytes. The Sodium Carbonate caused no change in many of the leucocytes, but others were broken up into adherent masses of granules like blood platelets. And the $CaCl_2$ broke up most of them into débris, but a few granular ones were unaffected.

This observation on the effect of peptone on extravascular blood is a serious obstacle to the acceptance of Wright's view of the ~~the~~ dissolution of the leucocytes within the vessels. Another difficulty consists in the observation of Bruce, to be presently mentioned, that the organs are abnormally crowded with leucocytes after injection of peptone. Possibly the conditions of the action of substances on blood withdrawn from the body are not quite the same as those obtaining when the blood is within the living vessels, but in view of the above observations it cannot be held as proven that any disintegration of leucocytes takes place within the vessels at any rate after the injection of peptone.

Werego (loc. cit.) examined the liver & spleen of rabbits killed during this stage, after the injection of carmine: and the liver, spleen & lungs of rabbits after the injection of B. anthracis. From these examinations, he concludes that the disappearance of the leucocytes immediately after the injection, is a mechanical effect of the introduction of solid particles (carmine or bacteria) into the blood, which are englobed by the leucocytes which then carry them to the internal organs. Here they are arrested, & especially in the liver, where they transmit their englobed particles to the endothelial cells of the organ. The relatively great disappearance of the polynuclear leucocytes is due to the fact that they alone (and not the lymphocytes) are capable of englobing solid particles. He states also that the injected materials are in part englobed directly by ~~the~~ other phagocytic cells, notably by the cells of the pulp of the spleen, & the endothelial cells of the liver.

Everard, Demoor & Massart (loc. cit.) point out that the bone-marrow must also be included in the loci of collection of the leucocytes, and that Werego's theory must be extended to the results of substances dissolved as well as particulate. In one of their experiments, after the injection into the peritoneal cavity, of a culture of B. metchnikovi previously filtered to get rid of the bacteria, there was a distinct hypoleucocytosis: while on the other hand the injection of living cultures of B. mycoïdes into

the peritoneum was followed by a much more feeble & transient diminution. They conclude that the microbic poison (like other poisons) accumulate probably in the liver, spleen & bone-marrow principally. Thus the hypoleucocytosis in the circulating blood is the necessary result of the afflux of white corpuscles to these parts: and as long as the microbes live and continue to secrete, the leucocytes are retained in these organs.

Goldscheider & Jacob (Zeitschrift f. klin. med. 1894 p. 373) examined the lungs of animals killed during this stage, & found that the capillaries & small vessels of these organs were crowded with leucocytes.

P. F. Müller (loc. cit.) examined the organs of his rabbits, killed during the period of marked leucocytopenia. He found the capillaries & small vessels of the lungs, liver, spleen, & in some the kidneys, to contain an excess of leucocytes, some of which contained incorporated microbes, but the majority were free from them. Some of the microbes were taken up by the endothelial cells of the organs.

J. L. Morse (Boston med. & Surg. Journal Mar. 7. 1895) made an autopsy on one rabbit killed during hypoleucocytosis following the injection of diphtheria toxine. He did not observe any increase in the leucocytes in the liver & kidney, but found the lung-capillaries crowded with them, whilst they were almost absent from the larger vessels of the lungs.

Bruce (Proc. Roy. Soc. Mar. 15. 1894) examined sections of various internal organs of rabbits, killed after injection of solution of peptone into the circulation, when the diminution of the circulating leucocytes was marked.
In all cases, he found a decided increase in the number of leucocytes present in certain of the organs, as compared with that in corresponding viscera from animals in which the number of circulating leucocytes was normal. This was especially the case with the lungs, which showed the greatest increase: next came the spleen: & then the liver. The other organs showed no particular change: the bone-marrow he does not mention, & apparently did not examine. It may be noted that he counted the polynuclear variety only.

From these various observations, it seems certain that the leucocytes which disappear do not dissolve in the blood, but accumulate in the internal organs. This, however, is only a partial explanation, since it becomes necessary to explain why it is that the leucocytes so rapidly transfer themselves from the blood to the internal organs. Some authorities attribute this, to "negative chemotaxis", consequent on the repellant action of the foreign substance, on the leucocytes. Gerard, Demoor, & Massart (loc. cit.) state that the polynuclear leucocytes are more chemotactic than the other varieties. But this explanation does not seem satisfactory: and it must be admitted that a really

good explanation is not at present forthcoming.
At the same time, it seems reasonable to suppose that when a foreign substance - either particulate or dissolved - is introduced into the blood, it should be at once taken up by the polynuclear granular leucocytes. Since these possess phagocytic properties in a high degree, much more so than any of the other varieties. The foreign substance will certainly induce metabolic changes in the protoplasm of the leucocyte which has taken it into its interior: and it is possible that one result of the metabolic changes will be an increased "stickiness" of the leucocyte, in consequence of which it will tend to adhere to the capillaries into which it passes: and this adhesion will continue until the capillary vessels & surrounding tissues are crowded with polynuclear leucocytes. This variety of leucocyte, according to Sherrington, is naturally more adhesive, as well as more amœboid, than the mononuclear. The result will be that the larger vessels will be comparatively devoid of polynuclear leucocytes, while there will be an apparent relative increase in the mononuclear.

In the case of leucocytopenia consequent on acute local inflammation, the diminution of the polynuclear granular cells in the general circulation may be very largely due to the amœboid character of these cells, which, as Sherrington suggests, will cause them to adhere in proportionately large numbers & escape in the vascular region of the local inflammation.

II Leucocytosis proper or Hyperleucocytosis

The numerical increase in the circulating ~~blood~~ leucocytes is usually more marked & of longer duration, than the preceding diminution. Its degree & rapidity of onset, however, vary with the nature & amount of the foreign substance introduced into the circulation. It is absent if the animal dies shortly after the injection, during the leucocytopenic stage; but if the animal survives long enough, it appears to be constantly present. Like the preliminary leucocytopenia, it comes on earlier when the injected substance is introduced directly into a vessel, than when it is injected subcutaneously.

Its occurrence after the injection of alkali-proteins & bacterial proteins has been investigated by Pörner (loc. cit.) who found that after the former, the leucocytosis lasted 24 hours, reaching its maximum in 8 hours & then declining: after the latter it lasted as long as 48 hours. This was after intravenous injection. After 3 or 4 injections repeated in the course of 24 hours, the leucocytosis was of very high degree, the white cells being to red as 1 to 38 in one case. In the highest degree, he notes that many of the leucocytes were arranged in groups & heaps.

After the injection of bacterial cultures (living, dead, and filtered) Everard, Denoor & Massart (loc. cit.) constantly observed hyperleucocytosis in all cases in which the animals survived. It was more marked

after the injection of virulent-cultures than after less virulent ones. The microbes used by them have been previously mentioned under the description of the leucocytopenia. Similar observations have been recorded by Kanthack & Hankin (loc. cit.), and Kanthack (loc. cit.). Experimental inflammatory leucocytosis has been studied especially by Sherrington (loc. cit.). The largest number of leucocytes met with per c.mm. of blood in his experiments was 55,000, the number at the outset of that particular experiment being 7,750. He states that in some of his experiments the leucocytosis was followed by a finial leucocytopenic phase before the normal condition was resumed.

Relative proportions of the different varieties of leucocytes during this stage.

The relative proportions of the mononuclear & polynuclear leucocytes is now in marked contrast with that which exists during the diminution stage: the former being diminished and the latter relatively increased. This change has been demonstrated by Löwit for the leucocytosis following injections of albumose, nuclein &c. In one of his experiments (on rabbits) the polynuclear were to mononuclear as 87 to 13. Hankin & Kanthack, Everard, Demoor & Massart note the same change after bacterial injections. The latter authors state that at the very commencement of the hyper leucocytosis,

it is not uncommon to find an abundance of vesicular (large mononuclear) leucocytes.
In inflammatory leucocytosis, Sherrington observed the proportion of granular (polynuclear) to hyaline (mononuclear) rise from 6·2 : 1 to 19·4 : 1 in one case: and he quotes Rieder as having seen the proportion rise to 20 : 1.
Sherrington (loc. cit.) has paid special attention to the behaviour of the coarsely granular leucocyte during the inflammatory leucocytosis of his experiments. This cell probably corresponds to Ehrlich's cell with a granulations (eosinophile). Sherrington finds that during the leucocytopenic stage, it, like the other granular cells, suffers numerical diminution: but when this stage passes off, and the total number of granular leucocytes in the blood becomes greatly increased, their increase is due to the "finely granular" cell entirely, and there is no accompanying increase of the "coarsely granular" cell. On the contrary, the number of the latter becomes still fewer, not merely in comparison with the rest of the granular, but also in proportion to the number of chromocytes, and, more striking still, absolutely as measured per unit-volume of the blood. He draws the conclusion that this cell is probably withdrawn from the general circulation, either by becoming fixed in some particular vascular region, or by passing out of the blood-vessels altogether.
He quotes Felsen as having noticed that in 3 cases

of croupous pneumonia, at the height of the fever. eosinophile cells appeared to be absent from the blood. Hoorden, on the other hand, has seen a great increase in the number of these cells in the blood in bronchial asthma at the time of the attack: and Canon has concluded that they are increased in number in the blood in all diseases of the skin.

Theories of Leucocytosis.

The changes in the organs during this stage, so far as they have been definitely observed, are limited to the continuance of the crowding of these parts with leucocytes, as during the leucocytopenic stage.

It was asserted by Körner (loc. cit.) that the blood of the veins contains a greater number of leucocytes than that of the arteries: & further that he found numerous leucocytes in process of division (amitosis) in the venous blood, but not in the arterial. He concluded that the proteins etc. introduced into the circulation, cause a "formative irritation" of the leucocytes, especially in the venous blood, which are stimulated to rapid division & multiplication. His statement that the leucocytosis is more marked in the venous, than in the arterial, blood, has been contradicted by Goldscheider & Jacob (Zeitschrift f. k. Med. 1894. xxv. 373.) and Kanthack (Brit. Med. Jour. June, 1892, p. 1301). Löwit's theory is that the leucocytes, when disintegrated during the

stage of "leucolysis", shed substances into the plasma which cause a chemical stimulation of the blood-forming organs, from which numbers of young cells pass into the blood and are converted into the polynuclear leucocytes, which now especially predominate. He supposes that this occurs in all forms of leucocytosis, from whatever cause. According to this theory (hyper)leucocytosis must always be preceded by hypoleucocytosis. Goldscheider & Jacob, however, by using repeated small injections, succeeded in causing a primary hyperleucocytosis - i.e. without any preliminary diminution. They also find that the lung-capillaries contain as many, or more leucocytes, during the hyperleucocytosis, than during the hypoleucocytosis. These authors conclude that the phenomena of leucocytosis are entirely due to "chemotaxic" action. The first effect of the injection is a repellent one on the leucocytes, which are arrested in the lung-capillaries, & there remain. The second is an irritative - attractive - action, on the blood-forming organs, and the white corpuscles already formed there are set free, and an increased formation of new cells takes place, which in their turn are also set free. Some of these are arrested in the lung-capillaries by continued repellant action. They explain death during hypoleucocytosis by continuation of repellant action, while the irritative action is so strong as to paralyse the blood-forming organs.

The hyperleucocytosis, without hypoleucocytosis,

occurring after small injections repeated at short intervals, they suppose to be due to the fact that each single dose is too small to exert much repellant action and accumulation of leucocytes in the lungs; but by their repetition at intervals the irritative action on the blood-forming organs gradually grows stronger and finally becomes much superior to the repellant-action of each single dose.

The theory of "chemotaxes" does not appear to explain leucocytosis ~~any~~ much more satisfactorily than it does the preceding diminution: and it seems hardly necessary to build up such a hypothesis. May it not be that the foreign substance, whether introduced from without, or being produced in an inflamed area by the inflammatory process itself, not only causes an increased "stickiness" of the polynuclear granular cells, but also comes into contact with the blood-forming organs & stimulates them to increased activity, the latter ~~effect~~ effect eventually gaining the predominance over the former? Sherrington suggests also, that since reproductive division of the leucocytes, inclusive of the polynuclear granular form, has now been shown to occur in the blood, the finely granular leucocytes may therefore increase in number by reproduction within the circulation as well as in the blood-forming organs.

Part II

The Action of Drugs on the Leucocytes of the intravascular Blood.

Changes in the Number of the circulating Leucocytes

During several months past I have been engaged in performing a series of experiments on the action of a number of drugs, on the changes in the number of leucocytes in the blood, & on the relative proportion of the different varieties present. These experiments have been carried on in the Pathological Laboratory of the University College, Liverpool, under the superintendence of Professor Boyce.

Method of Experiment.

The drugs were administered in all cases, by subcutaneous injection: and the animals used were exclusively rabbits. The drug was first dissolved, (or, when insoluble, suspended) in distilled water previously sterilised by boiling: (except in the case of one, viz. turpentine which was injected pure, without dilution). The injections were made with a syringe which was boiled thoroughly before each experiment. The site of injection was usually under the skin of the back or flank: probably the difference in the site of injection, and consequent difference in the rate of absorption, accounts for some variations in the rapidity of the blood changes, observed after experiments with doses of similar quantities of the same drug.

In ~~During~~ some of the earlier experiments, the animal was held while the injection was being made, but it was soon found that holding was not absolutely necessary, and in the later experiments, which form the majority of those to be recorded, it was dispensed with.
This seems to be of some importance in view of Löwit's statement, previously referred to, that simple binding down of an animal causes a diminution in the number of the circulating leucocytes.
The blood was examined at intervals after the experiment being obtained for this purpose, by pricking the animal's ear. The drop of exuded blood was taken up by the Thoma-Zeiss pipette and diluted with Toison's solution, the composition of which is as follows:— distilled water 160 c.cm: neutral glycerine 30 c.cm: sodium sulphate 8 grams: sodium chloride, 1 gram: methyl violet ·025 gram. (Römer, in Virchow's Archives. Band 128, p. 99). This solution tinges the leucocytes violet, & enables them to be easily picked out amongst the red corpuscles.
The leucocytes in the diluted blood were then counted on the Thoma-Zeiss slide. Those contained in the whole of the squares in 4 slides being counted in each case, so as to get a fair average. If the numbers in the 4 slides varied greatly, as it occasionally did, the counts were discarded & a fresh drop of blood taken & the count repeated. Very great care in the methods of examination is required to determine slight variation in the number of leucocytes, with proper accuracy.

The red corpuscles were enumerated in some of the experiments, but not in the majority.

The number of leucocytes per cubic millimetre of blood was calculated from the number contained in the squares, according to the usual formulæ.

Films of the blood on cover-glasses were also obtained by pricking the animal's ear. These were hardened by immersion for 2 hours in equal parts of absolute alcohol and ether: and then stained. The majority of the films were first stained with eosin - a saturated solution in glycerine being employed, & allowed to act for several hours: after washing in water & drying, they were next counterstained with hæmatoxylin for 10 minutes (Ehrlich's solution of hæmatoxylin, supplied by R. Kankack, Berners St., London, was found most satisfactory). Finally, after washing in water & drying again, the films were mounted in Canada balsam.

From the films, the percentage of mononuclear, transitional and polynuclear forms were estimated, by counting the proportion of these in different fields, 500 leucocytes being usually counted in each specimen.

In one or two ~~cases~~ experiments, a local inflammation was excited at the seat of injection: these cases are not recorded in the following tables. In all the recorded cases, therefore, it may be understood that no inflammatory reaction was detected, although in each case it was carefully looked for.

Before proceeding to the experiments it will be advisable to give an account of the leucocytes of the normal rabbit's blood, based for the most part, on my own observations of numerous samples.

Leucocytes of Normal Rabbit's Blood.

The number of leucocytes per cubic millimetre being influenced by food &c, a careful count was made & recorded, before each of the injections. As a rule, the number per cubic millimetre in a normal animal appears to be from 5,000 to 12,000.

Classification. Two varieties of leucocytes may be distinguished.

I Small mononuclear cells (often called lymphocytes). The size varies but the majority are not larger than the red corpuscles. The nucleus (like the cell-body) is spherical, & stains deeply. The protoplasm is very scanty: merely a rim around the nucleus of the smaller cells, but rather more abundant in the larger ones.

II Large mononuclear or "transitional" cells. These are distinguished from the first group, not only by their larger size, but also by the relatively large amount of protoplasm which surrounds the nucleus. At the same time there are all transitions between the cells of the two groups, and it is sometimes difficult to decide whether an individual cell should be included in group I or group II. The nucleus stains less deeply than that of the small mononuclear cell, & is often concave on the one side. The protoplasm colours faintly with strong solution of methylene blue: & as Kanthack & Hardy point out (Jour. of Physiology, Aug. 1894: p. 96), although it is usually regarded as

hyaline in character, a very high power of the microscope brings to view a number of very minute points which take up the basic stain. These authors suggest that the specific granules of the basophile granular leucocytes are the specialised homologues of these minute points. These cells vary in size but are all larger than the red corpuscles, and some appear to be the largest cells found in the blood.

III Large coarsely-granular eosinophile cells.

These form a relatively very small group but are well-defined from the others. Their size does not vary much in different individuals, being almost as large as the largest mononuclear. They are especially distinguished by the possession of a considerable number of large, spherical or slightly ovoid granules, of high refractive index, which have a special affinity for eosin & other acid dyes: these granules are embedded in an apparently structureless protoplasmic cell-substance. The nucleus is single, of irregular form, being often horse-shoe shaped, and stains rather feebly with basic dyes.

IV "Polynuclear" oxyphile granular cells.

In these cells, the nucleus is very irregular in shape, & often indeed appears to be really multiple, but it is said that the parts are all united by bands or filaments of nuclear substance. The nucleus stains more deeply than does that of the large eosinophile cells. The cell is usually somewhat larger than a red

corpuscle, and there is not a great difference in the size of different individuals. The cell-body contains a large number of very small spherical granules which crowd the otherwise clear, & optically structureless protoplasm. These granules are highly refractive, though less so than those of the preceding group of cells: and according to Kanthack & Hardy (loc. cit. p. 91) they have a higher refractive index as well as a greater oxyphile reaction than the corresponding granules in other animals. Further, these authors state that these two characters become more pronounced when the blood contains minimal amounts of microbic poison. With regard to the staining reaction of these granules in the rabbit, Ehrlich originally described them as staining with both acid and basic dyes, and hence called them amphophile. Kanthack & Hardy (loc. cit. p. 90) deny that they stain with basic dyes; while they take up acid stains in a marked degree.
My own observations entirely confirm the latter statement. I have found that the granules stain very readily & distinctly with eosin (in both glycerine & alcoholic solutions); and with acid fuchsin.

V Basophile granular cells. This is a very small group of cells, spherical in shape, and about the same size as those of the last group. The nucleus is irregular (less so than that of the oxyphile) & often trilobed. The protoplasm contains a very large number of minute granules which stain deeply with methylene blue.

Kanthack + Hardy (loc. cit. p. 95) state that the proportion of these cells, as well as the number + distinctness of their granules, becomes increased after a meal. Sherrington (Proc. Roy. Soc. Vol LV. p. 189) says that the basophile cells are so scanty that it is doubtful if they can be really considered as normal hæmic leucocytes.

Proportion of the above varieties. From the examination of numerous specimens of blood from different rabbits, I have come to the conclusion that the small mononuclear cells form about 38 per cent. of the total leucocytes, on the average: the large mononuclear + transitional forms, 2 to 4 per cent.: the large eosinophile, not more than 1 per cent.: the polynuclear granular (including both oxyphile + basophile) about 58 per cent. I have not specially observed the proportion of basophile cells alone: Kanthack + Hardy put it at 2 to 5 per cent. These authors give the percentage of small mononuclear as 70 to 80, and of polynuclear oxyphiles as 20 to 30. Bruce (Roy. Soc. Proc. Vol LV. p. 296) on the other hand, gives the percentage of these varieties as 31 + 51 respectively, while he gives the large mononuclear (under the name of "myelocytes") 16 per cent on the average.

No doubt this difference in the estimated percentage of the mononuclear + polynuclear forms is due to the fact that the proportions vary at different times and under different conditions. Food causes a marked increase in the small mononuclear forms,

and a corresponding relative diminution in the polynuclear oxyphiles.
All observers agree that the coarsely granular cells are relatively very scanty in the blood of the rabbit.

Experiments.

The experiments performed with each drug have been grouped together, and numbered under the heading of each drug.
The weight of the animal is noted before each experiment.
The date & hour of each observation of the blood, both before & after the injection, is given; as well as the time of the injection itself.
The first of the vertical columns on the right gives the total number of leucocytes (of all varieties) per cubic millimetre of blood.
The next 3 columns give the percentage (estimated from stained preparations) of (1) small mononuclear: (2) "transitional" and large mononuclear: (3) "polynuclear". The last group will include the granular oxyphile, eosinophile, & basophile forms. I have not estimated separately the percentage of large eosinophile, & basophile forms.
The last 3 columns give the ~~actual~~ estimated number per cubic millimetre of blood, of the ~~the~~ same 3 groups: the figures have been arrived at, not by counting of the leucocytes of the diluted blood itself, but by simple calculation from the percentages given in the other columns.

Iodide of Potassium

	Total leucocytes per c.mm	Percentage of Total number in a c.mm. of mono-nuclear	Trans-itional	Poly-nuclear	mono-nuclear	Trans-itional	Poly-nuclear
Expt 1. Weight 5 lbs							
May 7. 5 p.m.	10000	36	4	60			
5:30 p.m. 0.2 gram KI in 3 c.c. H_2O.							
— 8. 5 p.m.	10500	35	3	62			
Expt 2. Weight 4 lbs 14 oz.							
May 9. 4 p.m.	12000	40	3	57			
4.30 p.m. 0.5 gram KI in 3 c.c. H_2O							
— 10 4.30 p.m.	19700	34	3	63			
Expt 3 Weight 4 lbs 9 oz							
June 5. 11.30 a.m.	6250	30	6	64			
12.30 p.m. 1 gram KI in 5 c.c. H_2O							
1 p.m. } No symptoms observed	11458	17	6	77	1948	687	8910
4 p.m. }	17187	10	3	87			
— 6. 4 p.m. }	12500	26	4	70			
Expt 4. Weight 5 lbs							
June 19. 3 p.m.	9375	48	4	48	4500	375	4500
3.5 p.m. 1 gram KI in 5 c.c. H_2O							
4 p.m. } No symptoms	6250	56	16	28	3500	1000	1750
5 p.m. }	9375	44	4	52	4125	375	4875
— 20. 3 p.m. }	37500	45	3	52	16875	1125	19500
— 21. 12.30 p.m. }	5,500						

	Total leucocytes per c. mm.	Percentage of Mononuclear	Transitional	Polynuclear	Total number in a cmm. of Mononuclear	Transitional	Polynuclear
Expt. 5 Weight 5 lbs 4 oz.							
Dec. 23. 4 p.m.	12,500	41	3	56	5125	375	7000
4.15 p.m. 1 gram KI in 5 c.c. H_2O							
4.35 p.m.	18,300	28	2	70	5124	366	12810
4.55 p.m.	21,000						
5.20 p.m.	24,670	25	3	72	6168	740	17,762
— 24. 4 p.m.	16,000	25	1	74	4000	160	11,840

(4.35 p.m., 4.55 p.m., 5.20 p.m.: no symptoms)

Remarks. No coryza or other symptoms which this drug, in large doses, so commonly causes in man, were observed after any of the above experiments.

In Expts 1 + 2, no examination of the blood was made until 24 hours after the injection. After that interval there was practically no change in the number of leucocytes, in the case of Expt. 1: but Expt. 2, in which the dose was larger, shews a decided increase, with alteration in the ratio of mononuclear + polynuclear varieties.

Expt. 3 shews a distinct increase in the number of leucocytes half an hour after the injection of 1 gram, this being still more marked 2 hours later. At this time (4 p.m.) the number was nearly three times as great as before the experiment. 24 hours later still, there were twice the number before the experiment.

Expt. 4 is especially worthy of notice, inasmuch as it shews a distinct diminution (leucocytopenia) 55 minutes after the injection, with a contemporary increase in the

percentage of mononuclear forms. Why this diminution should be observed so long after the injection, while no decrease (but instead an increase) was noted 30 minutes after a similar dose in Expt. 3 + 5, is not clear: but its occurrence was undoubted, and it will be further noted in this Expt, that 24 hours after the injection the (hyper)leucocytosis was enormous, while in Expts 3 + 5 at this interval after the injection, the increased number was not nearly so marked as at an earlier period. Further, this Expt shews 2 days (45 hrs) after the injection, a drop in the number of leucocytes to below the number before the experiment. This is comparable to the diminution observed by Sherrington after the leucocytosis of acute local inflammation, in some of his experiments. This animal was not held during the injection. In all these 5 experiments, but especially Expt 3 + 5, the relative increase of polynuclear cells with diminution of mononuclear, co-incident with the increase in the total number of leucocytes, is very striking.

The "transitional" leucocytes appear to vary pretty much in the same line as the small mononuclear.

Antipyrin

		Total Leucocytes per c.mm.	Percentage of Mono-nuclear	Trans-itional	Poly-nuclear	Total number per c.mm. Mono-nuclear	Trans-itional	Poly-nuclear
Expt. 1. Weight 5 lbs.								
June 24. 3 p.m.		11.458	38	3	59			
3.30 p.m.	1 gram Antipyrin in 3 c.c. H_2O							
4 p.m.	animal greatly exhausted.	7291	45	3	52			
5 p.m.		3125	55	4	41			
— 25. 3 p.m.	Animal appears well.	19.531	22	2	76			
— 26. 3 p.m.		11.170						
Expt. 2. Weight 5 lbs.								
Dec. 19. 11.30 a.m.		13.000	39	3	58			
12 noon.	0.5 gram Antipyrin in 2 c.c. H_2O							
12.30 p.m.		21.500	42	6	52			
1 p.m.	Animal lively & apparently well.	24.000	32	2	66			
4.30 p.m.		24.000	38	3	59			
— 20. 12 noon		10.500	40	4	56			
Expt. 3. Weight 5 lbs.								
April 6. 2.45 p.m.		9000	35	3	62			
3.15 p.m.	1 gram Antipyrin in 3 c.c. H_2O							
3.45 p.m.	no symptoms observed	9000	45	3	52			
6 p.m.		25.000	25	3	72			
— 7. 3 p.m.			55	4	41			

	Total leucocytes per c.mm.	Percentage of mono-nuclear	Trans-itional	Poly-nuclear	Total number per comm. — of mono-nuclear	Trans-itional	Poly-nuclear
Expt. 4. Weight- 5 lbs 4 oz							
April 7. 12·30 p.m.	13,250	45	4	51			
1·7 p.m. 1 gram Antipyrin in 3 c.c. H_2O							
1·22 p.m.	13,400	68	2	30			
4·30 p.m. No symptoms	28,600	26	6	68			
— 8. 1 p.m.	14000						

Remarks. It will be observed that the injection of 1 gram of Antipyrin, in Expt. 1 was followed by a very marked diminution in the number of leucocytes, pronounced 1/2 hour after the injection, but very striking indeed 1 1/2 hour after: while, on the other hand, a similar dose in Expts. 3 & 4, was followed, in the one case 1/2 hour after & in the other 15 minutes after, by practically no change in the total number. This apparent want of agreement in the result of the 3 experiments, appears to me to be due to the fact that the rabbit in Expt. 1 was held down on the table, lying on its side, by an assistant, during the injecting of the solution. When it was released it lay on its belly with legs extended, evidently much exhausted, & with rapid breathing. This condition continued for an hour, and the rabbit did not seem well for 2 hours after the experiment. It will be observed that the diminution of the number of leucocytes coincided with this period of exhaustion.

Löwit, as previously stated, has observed a diminution

to occur in animals when bound down, and I think I am justified in regarding the marked leucocytopenia of this experiment as due to a large extent to the fact that the animal was held with unnecessary force by the assistant. The animals of Expts 3 & 4 were not held at all, & no symptoms of exhaustion followed the injections. Although, in Expts 2, 3, & 4, no diminution was observed in the total number of leucocytes per cubic millimetre, the figures in the "percentage" columns shew that in each experiment there was a more or less marked relative increase of mononuclear cells within half an hour after the injection. Later, in all the expts there was the relative diminution of mononuclear, & increase of polynuclear forms, which appears to characterise (hyper) leucocytosis.

J. Horbaczewski (quoted in Sajous' Annual of the Universal Medical Sciences, 1893. Vol 5. p 87) from a number of observations concludes that Antipyrin, in doses of 2 grammes, causes an increase in the number of leucocytes in the blood, & a diminution of the uric acid eliminated in the urine.
The diminution in the uric acid eliminated, he explains (Halliburton's Lectures - loc. cit.) as being due to the leucocytes disintegrating very slowly after the administration of Antipyrin. It is an exception to the general rule that leucocytosis is accompanied by increased elimination of uric acid.

Carbolic Acid

	Total number of leucocytes per cmm.	Percentage of Mono-nuclear	Trans-itional	Poly-nuclear	Total number in a cmm. — of Mono-nuclear	Trans-itional	Poly-nuclear
Expt 1. Weight 4 lbs 12 oz.							
May 30. 3 p.m.	9375	55	2	43			
3·10 p.m. 0·15 gram Carbolic Acid dissolved in 2 c.c. Water							
3·55 p.m.	7812	70	3	27			
5 p.m.	16000	30	2	68			
— 31. 4 p.m.	7812	44	4	52			
Expt 2. Weight 4 lbs 14 oz.							
Jan. 15. 12 noon	11,000	50	3	47			
12·10 p.m. 0·1 gram Carbolic Acid dissolved in 2 c.c. H_2O							
12·40 p.m.	10750	56	4	40			
4·20 p.m.	18250	21	4	75			
— 16. 4 p.m.	10750						
Expt 3. Weight 5 lbs 4 oz.							
April 13. 3.15 p.m.	11251	42	3	55	4725	338	6188
4·18 p.m. 0·3 gram Carbolic Acid dissolved in 5 c.c. H_2O							
4·30 p.m.	9357	60	3	37	5614	286	3457
5·18 p.m.	14500	47	4	49	6815	580	7105
— 14. 3·30 p.m.	23000	35	3	62	8050	690	14260

Remarks. No symptoms were observed after the injection in any of these experiments. All threw a diminution of the total leucocytes shortly after injection: followed by a distinct increase, though not by any means of the same degree as after some other drugs. Note corresponding change in percentage of different forms.

Digitalin

	Total leucocytes per cmm.	Percentage of mononuclear	Transitional	Polynuclear	Total number per cmm. of mononuclear	Transitional	Polynuclear
Expt. 1. Weight- 5 lbs							
Dec. 5. 3 p.m.	17500	35	3	62			
3·15 p.m. 1/45 grain Digitalin in 1 c.c. H_2O							
4·15 p.m.	12500	42	3	55			
— 6. 4 p.m.	33,300	24	2	74			
Expt. 2. Weight- 5 lbs 4 oz.							
Apr. 4. 3·30 p.m.	9750	48	4	48			
4 p.m. 1/40 grain Digitalin in 1 c.c. H_2O							
4·30 p.m.	7343	52	2	46			
5 p.m.	12400	30	3	67			
— 5. 4 p.m.	18600						

Remarks. No symptoms followed the injection of this powerful drug. The digitalin used was the ordinary commercial form.

Both experiments shew a stage of total diminution with relative increase of mononuclear cells, followed by a marked increase, with relative great increase of polynuclear as compared with mononuclear forms.

Camphor

		Total Leucocytes per c.mm.	Percentage of Mono-nuclear	Trans-ition'l	Poly-nuclear	Total leucocytes, c.mm. of Mono-nuclear	Trans-ition'l	Poly-nuclear
Expt. 1.	Weight - 4 lbs 14 oz.							
Aug. 26.	12·15 p.m.	4500	38	3	59			
	12·30 p.m. 0·2 gram Camphor (pulverised & suspended in H_2O)							
	1·30 p.m.	4000	46	4	50			
	4·30 p.m.	12500	24	5	71			
— 27.	3·30 p.m.	10,000	32	4	64			
Expt. 2.	Weight - 5 lbs 2 oz.							
April 4.	11 a.m.	7600	44	4	52			
	11·30 a.m. 0·4 gram Camphor pulverised & suspended in 6 c.c. H_2O							
	12·30 p.m.	5200	54	4	42			
	4 p.m.	22000	23	6	71			
— 5.	12 noon	12300	30	5	65			

Remarks. The remarks under the experiments with Digitalin, as to the effect on the leucocytes, are equally applicable to the effect of Camphor. No symptoms were observed in the experiments with this drug.

Turpentine

	Total leucocytes per cmm	Percentage of mononuclear	Transitional	Polynuclear	Total number per cmm of mononuclear	Transitional	Polynuclear
Expt. 1. Weight 4 lbs 14 oz							
Nov. 1. 12.30 p.m.	9500	48	5	47			
1 p.m. 0.5 c.c. Turpentine							
3.45 p.m.	8500	58	5	37			
— 2. 12 noon	6000	60.5	4	35.5			
Expt. 2. Weight 4 lbs 12 oz.							
Nov. 7. 3.30 p.m.	10,000	53	5	42			
4 p.m. 1 c.c. Turpentine							
5 p.m.	9500	64	6	30			
— 8. 12 noon	6400	68	9	73			
Expt. 3. Weight 5 lbs 8 oz.							
March 24. 12.15 p.m.	6000	54	4	42			
12.30 p.m. 2 c.c. Turpentine							
1 p.m.	5000	61	5.5	33.5			
3.30 p.m.	15750	35	1	64			
— 25. 3 p.m.	5000						
Expt. 4. Weight 5 lbs 2 oz.							
March 26. 12 noon	6000	46	4	50			
12.5 p.m. 2 c.c. Turpentine							
12.35 p.m.	5200	58	5	37			
4.35 p.m.	27500	21	3	76			
— 27. 12 noon	7000						

	Total leuco. cytes per c.mm.	Percentage of			Total number per		
		mono-nuclear	Trans-itional	Poly-nuclear	mono-nuclear	trans-itional	Poly. nuclear
Expt. 5. Weight- 5 lb 4 oz.							
April 2. 3·30 p.m.	13,500	54	2	44			
4·15 p.m. 1 c.c. Turpentine							
4·30 p.m.	12,000	56	3	41			
5·30 p.m.	18,600	30	2	68			
Expt. 6. Weight- 5 lb 8 oz.							
April 3. 3·15 p.m.	12,500	48	3	49			
3·45 p.m. 1 c.c. Turpentine							
4·15 p.m.	8,000	63	2	35			
5 p.m.	17,600	23	3	76			
—— 4. 3·45 p.m.	10,200						

Remarks. No ill effects were observed in the animals after any of these injections, so large a dose as 2 c.c. apparently causing the rabbit no inconvenience. The turpentine was injected pure + undiluted.

In all the experiments, the diminution in the number of circulating leucocytes was evident shortly after the injection; the degree of diminution was not observed to correspond with the dose but possibly this was owing to the blood examination not corresponding in time with the point of greatest diminution. Expt. 6, in which the dose was 1 c.c., shews half an hour after the injection, a relatively greater decrease, than do Expts. 3 + 4 at a similar interval after doses of 2 c.c. of the drug.

Another probable factor in the difference in these (+other) experiments in which the doses were similar, lies in the different rate of absorption of the injected substance from the subcutaneous tissue into the blood. The rapidity of absorption varies with the site of injection, & probably is influenced by other conditions.

Corresponding with the total diminution per cubic millimetre, there is the usual increase in the percentage of the mononuclear forms.

At a later period, there was observed, in Expts 3, 4, 5, & 6, a marked increase in the total leucocytes per cubic millimetre, with increase in the percentage of the polynuclear variety. The leucocytosis was observed to be more marked in degree, after the injections of 2 cc. than after those of 1 c.c.

The records of Expts 1 & 2, shew no increase, probably because examinations of the blood were not made at a time when this phenomenon was likely to be present.

A slight fall to below the number before the injection, was recorded on the day after the injection, in 4 out of 5 experiments in which the blood was examined at the latter period.

Thyroid Gland Tabloids

		Total leucocytes per c.mm.	Percentage of mono-nuclear	Trans-animal	Poly-nuclear	Total leucocytes per c.mm. — of mono-nuclear	Trans-animal	Poly-nuclear
Expt. 1.	Weight - 5 lbs 4 oz.							
June 26.	12 noon	6075	46	2	52			
	12.15 p.m. 2 Tabloids powdered + suspended in H_2O (5 c.c.)							
	1.15 p.m.	7031	44	3	53			
	5 p.m.	22,500	19	4	77			
— 27.	3 p.m.	6875	42	4	54			
Expt. 2.	Weight - 5 lbs							
Nov. 27.	12.30 p.m.	6,660	52	3	45			
	1 p.m. 2 Tabloids, powdered + suspended in 10 c.c. H_2O							
	4 p.m.	5,660	29	2	69			
— 28.	3.30 p.m.	23,000	46	2	52			
— 29.	4 p.m.	16,000	67	2	31			

Remarks. The Tabloids used were those of Messrs Burroughs. Wellcome & Co: each of them represents 5 grains of sheep's thyroid, & they are said to be prepared by drying the gland at a low temperature, powdering, & compressing the powder together with a small quantity of sodium chloride, into the tabloid form. In the experiments the tabloids were first reduced to fine powder, which was then ~~suspended~~ shaken up with water & injected through a needle of large calibre. No particular effect was noticed in the animals after the injections. The blood, in the case of Expt. 1, showed a slight increase of leucocytes one hour after, which

had become very marked 3¾ hours later still; while 27 hours after the injection the number was practically normal. Accompanying these changes were the usual percentage alterations of the mononuclear + polynuclear varieties, as with preceding drugs.

The record of Expt. 2. differs in exhibiting a distinct diminution in the number of leucocytes, as late as 3 hours after the injection: whilst the increase appears to have been delayed also, as compared with that of Expt. 1; being very marked 27 hours after the injection: it also seems to have been of much longer duration, since an examination of the blood 2 days after the injection shews a still very pronounced leucocytosis. The percentages of Expt. 2, at the time of the leucocytopenia, differ from those of all the previously recorded experiments in exhibiting a marked diminution of mononuclear, with relative increase of polynuclear, leucocytes. This apparent anomaly I can only conclude must have been due to some error in the counting.

Goldschieder + Jacob, (Deutsch. Med. Wochen. Aug. 9. 1894) state that extract of thyroid gland, like that of the liver, pancreas + kidney, have no influence on leucocytosis: while extracts of spleen, bone-marrow, + thymus cause first a great diminution, then a marked increase, of the leucocytes.

My experiments with Thyroid Gland tablets appear to contradict their statement of the effect of thyroid extract: but I am not aware of their method of preparing the extract: very probably the difference of the preparation accounts for the difference in the result.

Salicine

	Total Leucocytes per cmm.	Percentage of mononuclear	Transitional	Polynuclear	Total per cmm. of mononuclear	Transitional	Polynuclear
Expt. 1. Weight- 5 lbs							
Oct. 23. 12.30 p.m.	10,000	36	4	60			
1 p.m. 0.3 gram Salicine in 5 c.c. H_2O							
3.30 p.m.	6428	56	6	38			
— 24. 3.30	9000	40	2	58			
Expt. 2. Weight- 4 lbs 14 oz.							
Apr. 9. 3.15 p.m.	10,000	40	4	56			
4.20 p.m. 1 gram Salicine in 15 c.c. H_2O							
5.10 p.m.	8,200	62	4	34			
6 p.m.	14,250	59	3	38			
— 10. 4.20 p.m.	20,000	34	2	64			

Remarks. No symptoms observed after either experiment. Expt. 1 shews a considerable diminution 2½ hours after the injection. No increase observed but the blood was not examined until next day.

Expt. 2, in which the dose was more than three times that in Expt. 1, shews a distinct diminution 50 minutes after the injection. 50 minutes later there was a distinct though not great increase, which was more marked 24 hours after the injection.

Salicylate of Sodium

		Total Leucocytes per c.mm.	Percentage of			Total per c.mm. of		
			mono-nuclear	Trans-ilional	Poly-nuclear	mono-nuclear	Trans-ilional	Poly-nuclear
Exp^t 1.	Weight- 5 lbs 6 oz.							
May 28.	11 a.m.	6250	36	3	61			
	11·30 a.m. 1 gram Salicylate of Sodium in 4 c.c. H_2O							
	12·30 p.m.	4500	48	4	48			
	3 p.m.	7300	40	2	58			
— 29	4·30 p.m.	4687	41	2	57			
Exp^t 2.	Weight- 5 lbs 2 oz.							
Jan. 7.	11·45 a.m.	14.000	44	2	54			
	12 noon. 1 gram Salicylate of Sodium in 5 c.c. H_2O							
	12·30 p.m.	8000	58	4	38			
	4·10 p.m.	14000	49	1	50			
— 8.	3·30 p.m.	15000	47	3	50			

Remarks. In both of the experiments with this drug, the dose was of similar amount, & in neither case were any symptoms observed to follow the injection. Both shew a very distinct diminution: observed in Exp^t 1, an hour after, and in Exp^t 2, half an hour after, the injection. This was followed in both by an increase, as the tables shew, but the increase was very slight in degree, & in the case of Exp^t 2 was so long after the injection that it cannot be considered certain that such a slight increase had any connection with the experiment at all. Even in Exp^t 1, the increase was not

very pronounced at the time of the examination 3½ hours after the injection: and in Expt. 2, 4 hours + 10 minutes after injection, the number exactly coincided with that before the experiment.

In fact it may be said that the principal change in the blood in these experiments was a marked diminution in the number of leucocytes per cubic millimetre: with a co-incident relative increase of the mononuclear (and transitional) forms & a corresponding diminution of the polynuclear variety. At the same time, the occurrence of a succeeding increase, though slight in degree, with a co-incident shifting of the percentages in the opposite direction, is important as shewing that the experiments with this drug are not exceptions to the general rule as to the variations in the number & proportions of the leucocytes, which has been observed all through this series of drug injections.

Quinine Hydrochlorate

	Total leucocytes per cmm.	Percentage of mono nuclear	Trans	Poly nuclear	Total number per cmm. of mono nuclear	Trans	Poly nuclear
Expt. 1. Weight- 5 lbs							
Sep. 27. 12·30 p.m.	6,000	40	4	56			
1 p.m. 0·2 gram Quinine hydrochlorate in 5 c.c. H_2O							
2 p.m.	4240	65	6	29			
4 p.m.	15,200	31	2	67			
— 28. 12 noon	12,500	34	2	64			
— 29 12 noon	7000						
Expt. 2. Weight- 4 lbs 12 oz.							
Jan. 1. 12 noon	13,500	35	3	62			
12·15 p.m. 0·4 gram Quinine hydrochlorate in 10 c.c. H_2O							
12·45 p.m.	7500	44	5	51			
2 p.m.	6000	45	3	52			
4·30 p.m.	10,000	46	4	50			
— 2. 4·15	10,000	56	2	42			
Expt. 3. Weight- 4 lbs 14 oz.							
April 16. 12·15 p.m.	4500	41	4	55			
12·30 p.m. 0·6 gram Quinine hydrochlorate in 20 c.c. H_2O							
1 p.m. (animal seems ill)	3571	50	3	47			
4·30 p.m. (very ill)	2500	62	5	33			
— 17. 4·30 p.m. (Appears to be recovering)	3000	46	4	50			
— 18. 12·30 p.m. (Eating badly + very quiet)	3500	52	3	45			
— 19. 11 a.m. (Found dead in its stall)							

No gross lesions discovered post-mortem.

Remarks. The injections of Expts 1 & 2 were followed by no symptoms. That of Expt. 3, the dose being larger, was followed by a fatal result 3 days later. Shortly after the injection, in the last experiment, the animal developed symptoms of a nervous character. Its breathing at first was rapid & shallow but soon became abnormally slow. It lay on its side & seemed unable to stand: when it attempted to get up, it stumbled blindly about. The symptoms had disappeared by next day, but the animal continued to be dull, & ate badly; and on the third morning after the injection it was found dead.

Expt. 1 shows first a distinct diminution of leucocytes after the injection, followed by an increase.

Expt. 2. shows a diminution of greater degree & longer duration, and no increase was observed, though it may possibly have occurred between the last examination on Jan 21st, & that on Jan 22nd, 24 hours later.

Expt. 3, shows a remarkable & apparently progressive diminution up to the day before the animal's death. Here probably no increase occurred at all, the rabbit dying during the leucocytopenic stage. This is analogous to the effect of virulent microbic injections, after which death sometimes occurs before the diminution stage has passed off. A remarkable feature in this experiment is the long continuance of the leucocytopenia.

Sulphate of Atropine

	Total Leucocytes per c.mm.	Percentage of Mono nuclear	Trans itional	Poly. nuclear	Total number per c.mm. of Mono nuclear	Trans itional	Poly. nuclear
Expt. 1. Weight-							
July 18. 12 noon	7000	36	4	60			
1 p.m. 0.05 gram Atropine Sulphate in 1 c.c. H_2O							
*2 p.m.	6000	48	4	48			
4 p.m.	10,000	28	2	70			
— 19. 3 p.m.	15,000						
Expt. 2. Weight-							
Oct. 29. 12.30 p.m.	10,000	38	4	58			
1 p.m. 0.05 gram Atropine Sulphate in 1 c.c. H_2O							
1.30 p.m.	9,330	55	5	40			
4 p.m.	12,000	30	3	67			
— 30. 4 p.m.	6000	37	6	57			
Expt. 3. Weight-							
June 11. 12.30 p.m.	10,000	44	4	52			
1 p.m. 0.15 gram Atropine Sulphate in 1 c.c. H_2O							
1.30 p.m.	8420	54	4	42			
4.30 p.m.	16400	32	3	65			
— 12. 1 p.m.	11,400	47	3	50			
Expt. 4. Weight- 5 lbs							
Dec. 17. 11.30 a.m.	8670	47	2	51			
12 noon 0.2 gram Atropine Sulphate in 2 c.c. H_2O							
12.20 p.m.	7330	64	3	33			

		Total Leucocytes per c.mm.	Percentage of Mononuclear	Transitional	Polynuclear	Total number per c.mm. of Mononuclear	Transitional	Polynuclear
Dec. 17.	1 p.m.	4000	68	4	28			
	4.30 p.m.	6800	40	3	57			
— 18.	12 noon	11,500	18	2	80			
Expt 5.	Weight - 5 lbs 4 oz.							
April 22.	12.30 p.m.	13000	37	3	60			
	1 p.m. 0.3 gram Atropine Sulphate in 2 c.c. H_2O							
	2 p.m.	8420	50	4	46			
	5 p.m.	13,000	46	4	50			
— 23.	1 p.m.	18500	22	3	75			

Remarks. No symptoms, except slight dilatation of the pupil, were noted in these experiments.

In the blood, in the case of all the experiments, the injection was followed by a marked diminution in the number of leucocytes, which appeared to continue for a relatively long time, & was accompanied by a rise in the percentage of mononuclear cells. The leucocytosis which was observed later, after all the experiments, was a much less evident phenomenon, & was relatively delayed considerably: in both these features - its slight degree, & comparatively late onset - it contrasts with the leucocytosis following the injection of Potassium Iodide, Antipyrin, & some other of the drugs of this series, which are rapidly followed by a leucocytosis of high degree. In this case, however, as in the others, it was associated with a relative diminution of mononuclear & increase of polynuclear forms.

Expt 7

		Total Leucocytes per cmm	Percentages of Mono-nuclear	Trans-cleinal	Poly-nuclear
Feb. 19. 11.55 a.m.	Weight-5 lbs	9000	35	4	61
12 noon. 0.3 gram Atropine Sulphate in 3 c.c. H_2O					
1 p.m.		17,000	25	3	72
4.30 p.m.		20,000	20	1	79
— 20. 3.30 p.m.	Weight-5 lbs	16.000			
4 p.m. 0.3 gram Atropine Sulphate in 3 c.c. H_2O					
4.20 p.m.		12,800			
— 21. 12.30 p.m.	Weight-4 lbs 13 oz	15.000	27	5	68
1 p.m. 0.3 gram Atropine Sulphate in 3 c.c. H_2O					
4.15 p.m.		25,500	11	3	86
— 22. 3.15 p.m.	Weight-4 lbs 14 oz	16500	32	4	54
3.45 p.m. 0.3 gram Atropine Sulphate in 3 c.c. H_2O					
4.20 p.m.		12850	42	8	50
— 23. 12.15 p.m.	Weight-4 lbs 12 oz	20 000	21	4	75
12.45 p.m. 0.3 gram Atropine Sulphate in 3 c.c. H_2O					
1 p.m.		18500	28	5	67
— 24. 3.45 p.m.	Weight-5 lbs	17000	26	2	72
4.30 p.m. 0.3 gram Atropine Sulphate in 3 c.c. H_2O					
4.40 p.m.		14000	36	3	61
— 25. 3.15 p.m.	Weight-4 lbs 15 oz	21000	29	6	65
4.5 p.m. 0.3 gram Atropine Sulphate in 3 c.c. H_2O					
4.35 p.m.		17000	46	6	48

The rabbit during the whole course of this experiment took its food well & appeared as lively & well as usual.

Remarks on Expt. 7. This experiment, as will be seen from the table, consisted in a series of 7 separate injections; each injection was of 0·3 gram of Atropine Sulphate. One injection was given each day for a week; the time of day of the injection, as well as of the blood examination, varied for reasons of convenience. The primary object of the experiment was to observe the effect of atropine, (or absence of effect), on the structure of the granular leucocytes, in which connection it will be again referred to: but the numbers of the leucocytes, & of the varieties, are recorded here for the purpose of exhibiting their oscillations under the conditions of such a series of injections. The single dose was larger than that used in any of the preceding experiments with this drug (except Expt. 6) without going into details which have been already given, it may be stated that the effect of each single injection, ~~was~~ on the number, & percentage of the varieties, of the leucocytes, was quite analogous to the effect of each of the Expts 1 to 6: with this difference, that the diminution stage was less marked after each of the injections, as well as of shorter duration, than in Expts 1 to 6. On the other hand, the stage of increase was more marked, & came on earlier. In the case of the first injection of the series, curiously enough, the blood an hour after the injection showed a marked leucocytosis, instead of the diminution one would have expected from the results of Expts 1 to 6.

Maurel (quoted in Sajous Annual of the Universal Medical Sciences, 1893 Vol V. 5-7) has observed that- 5 centigrammes of atropine when added to 100 grammes of human blood, instantly kills all the leucocytes contained in it: and the addition of 2 centigrammes to 100 grammes of blood kills the leucocytes in a few hours. On the other hand he found that- the leucocytes contained in 100 grammes of rabbit's blood were not- affected by 2 centigrammes of atropine.

According to J. Norbaczewski, atropine, in daily doses of 1 milligramme, produces a lessening in the number of leucocytes in the blood, & also a diminished excretion of uric acid by the kidneys.

Pilocarpin

	Total leucocytes per cmm	Percentage of Mono-nuclear	Trans-ition	Poly-nuclear			
Expt 1. Weight - 4 lbs 14 oz.							
July 1. 11.30 a.m.	5320	46	3	51			
12 noon. 0.01 gram Pilocarpin Nitrate in 1 c.c. H_2O							
1 p.m. (Salivation profuse)	7272	47	3	50			
4.30 p.m. (Salivation ceased)	41,904	16	3	81			
— 2. 12 noon	14,400	36	4	60			
— 3. 4 p.m.	11,000						
Expt 2. Weight - 5 lbs.							
Dec. 13. 11.30 a.m.	12500	39	2	59	4875	250	7375
12 noon. 0.01 gram Pilocarpin Hydrochlorate in 1 c.c. H_2O							
12.20 p.m. (Salivation profuse)	8500	55	2	43	4675	170	3655
1 p.m. (Salivation profuse)	13,500	46	1	53	6210	135	7155
4 p.m. (Salivation ceased)	36,000	9	1	90	3240	360	32400
— 14. 12 noon	9000	44	3	53	3960	270	4770

Remarks. In each of these experiments, the injection was followed immediately by salivation. This symptom was noticed within 1 or 2 minutes after the withdrawal of the needle: & continued profusely for about an hour. In Expt 1, the blood was not examined until an hour after the injection, & there was then a slight increase. In Expt 2, twenty minutes after injection, the diminution was pronounced, but at the end of an hour it was replaced by an increase. 3 hours later still, the increase was very great indeed, in both experiments. There is the usual change in the percentage of mononuclear & polynuclear forms.

Expt 3.

	Total leucocytes per c.mm	Percentage of mono-nuclear	trans-ichimal	poly-nuclear			
Jan. 20. 5 p.m. Weight 4 lbs 6 oz.	8000	38	5	57	3040	400	4560
— 21. 12·30 p.m. 2 grains Pilocarpin nitrate in 4 c.c. H_2O							
1 p.m.	9.300	66	4	30	6138	372	2790
4·20 p.m.	40.000	14	3	83	5600	1200	33200
— 22. 3 p.m. Weight 4 lbs 8 oz.	22.500	28	4	68	6300	900	15300
4 p.m. 2 grs Pilocarpin nitrate in 4 c.c. H_2O							
4·30 p.m.	16 000	60	6	34	9600	960	5440
— 23. 3·30 p.m. Weight 4 lbs 6 oz.	23.500	25	4	71	5875	940	16,685
3·50 p.m. 2 grs Pilocarpin nitrate in 4 c.c. H_2O							
4·40 p.m.	15,000	42	10	48	6300	1500	7200
— 24. 11·50 a.m.	23,000	33	7	60	7590	1610	13,800
12 noon. 2 grs Pilocarpin nitrate in 4 c.c. H_2O							
1 p.m.	14,000	42	4	54	5880	560	7560
4·30 p.m.	60,000	19	8	73	11400	6400	43800
— 25. 12·45 p.m. Weight 4 lbs 8 oz.	27,500	29	4	67	7975	1100	18425
1 p.m. 2 grs Pilocarpin nitrate in 4 c.c. H_2O							
3·15 p.m.	70, 000				11200	7000	51800
4·30 p.m.		16	10	74			
— 26. 1 p.m. 2 grs Pilocarpin nitrate in 4 c.c. H_2O							
— 27. 12·30 p.m.	20,000	31	5	54	6200	1000	10800
1 p.m. 2 grs Pilocarpin nitrate in 4 c.c. H_2O							
4 p.m. Weight 3 lbs 14 oz.	50,000	9	5	86	4500	2500	43000
— 28. Killed.							

Expt. 4.

			Total leucocytes per cmm.	Percentage of mono-nuclear	Trans-itional	Poly-nuclear
March 8.	3 p.m.	Weight-5 lbs 8 oz.	13,500	50	4	46
	3·30 p.m.	2 grains Pilocarpin nitrate in 4 c.c. N_2O (some bad)				
	3·45 p.m.		12,500	45	5	50
— 9.	3·30 p.m.		16,000	26	4	70
	4·15 p.m.	2 grs Pilocarpin nitrate in 4 c.c. N_2O				
	4·45 p.m.		17000	39	5	56
— 10.	3·50 p.m.	Weight-5 lbs 5 oz.	40,000	21	3	76
	3·55 p.m.	2 grs Pilocarpin nitrate in 4 c.c. N_2O				
	4·10 p.m.		24000	42	4	54
	4·25 p.m.		30 000	30	3	67
	4·55 p.m.		42,000	20	2	78
— 11.	3·50 p.m.	Weight-5 lbs 4 oz.	30,000	24	3	73
	4 p.m.	2 grs Pilocarpin nitrate in 4 c.c. N_2O				
	4·10 p.m.		16500	48	4	48
	4·30 p.m.		25600	34	4	62
	5 p.m.		36,000	21	5	74
— 12.	3·50 p.m.	Weight-5 lbs 2 oz.	32,000	37	9	54
	4 p.m.	2 grs Pilocarpin nitrate in 4 c.c. N_2O				
	4·30 p.m.		30,000	55	3	42
	5·10 p.m.		35,200	28	1	61
— 13.	12·30 p.m.	Weight-5 lbs	22,400	43	1	56
	12·45 p.m.	2 grs Pilocarpin nitrate in 4 c.c. N_2O				
	12·52 p.m.		14200	55	1	44
	3·30 p.m.		35600	22	4	74
	5 p.m.		46,420	12	3	85

		Total leucocytes per cmm	Percentage of mono-nuclear	Trans-ilional	Poly-nuclear
March 14. 3.15 p.m.	Weight-5 lbs	25200	37	3	60
3.30 p.m. 2 grs Pilocarpin hcl in 4 c.c. H_2O					
5.15 p.m.		41,000	21	2	77
—— 15. 12.55 p.m.	Weight-5 lbs 3 oz.	30200	34	2	64
1 p.m. 2 grs Pilocarpin hcl in 4 c.c. H_2O					
—— 16. 12.45 p.m.	Weight-5 lbs 2 oz.	34000	36	2	62
1 p.m. 2 grs Pilocarpin hcl in 4 c.c. H_2O					
3.30 p.m.		40500	17	3	80
5.15 p.m.		51,200	16	2	82
—— 17. 12.30 p.m.	Weight-5 lbs 3 oz.	22600	38	3	59
1 p.m. 2 grs Pilocarpin hcl in 4 c.c. H_2O					
5 p.m.		42700	20	1	79
—— 18. 4.25 p.m.	Weight-5 lbs 2 oz.	24600	32	2	66
4.30 p.m. 2 grs Pilocarpin hcl in 4 c.c. H_2O					
—— 19. 3.25 p.m.	Weight-5 lbs	26400	33	3	64
3.30 p.m. 2 grs Pilocarpin hcl in 4 c.c. H_2O					
3.45 p.m.		18600	42	4	54
—— 20. 12.15 p.m.	Weight-5 lbs	31,300	35	2	63
12.20 p.m. 2 grs Pilocarpin hcl in 4 c.c. H_2O					
12.50 p.m.		30,000	60	3	37
—— 21. 3.20 p.m.	Weight-4 lbs 14 oz.	36,000	30	2	68
3.30 p.m. 2 grs Pilocarpin hcl in 4 c.c. H_2O					
4 p.m.		28,400	51	3	46
4.30 p.m.		40550	25	2	73
5 p.m.		47300	19	2	79
—— 23. 3.30 p.m.	Weight-5 lbs 2 oz.	13,000			

Remarks on Expts 3 & 4.

The primary object of these experiments was to determine if any change occurred in the structure of the granular leucocytes under the influence of very large & repeated doses of this drug. The results of the observations bearing on this question will be referred to later.

The numbers of the leucocytes & the percentage of the varieties, at different intervals after the injections, have been carefully observed & recorded: and they exhibit a series of changes relative to each single injection, similar to those observed in Expts 1 & 2 with pilocarpin.

In Expt. 3, the injections were given daily for 7 days: in Expt. 4, daily for 14 days. Each injection was followed by immediate & profuse salivation which continued from one to two hours & then passed off.

Sweating of the feet was also observed shortly after the injection & continuing somewhat later than the salivation.

The animals appeared to be somewhat exhausted, with rapid breathing, for 20 to 30 minutes after each injection: but soon recovered & ate well. It seems remarkable that the daily repetition of such large doses, followed as they were by very profuse salivation & perspiration, should have caused so little loss of body-weight, or other persistent ill effect.

The degree of leucocytosis after some of the injections was very high indeed: and it will be noted that the number was always quite much above the normal when

the animal received the next injection. The further rapid fall after an injection, with relative increase of the mononuclear cells, was usually very distinct- when the blood was examined early enough after the injection.

The action of Pilocarpine on the leucocytes of escharvascular human blood has been studied by Maurel (quoted in Sajous Annual of the Universal Medical Sciences Vol V. 1893. B-43) He found that- 10 centigrammes of the drug was sufficient- to destroy the leucocytes contained in 100 grammes of blood: 5 centigrammes added to a similar quantity of blood, permitted the leucocytes to live a few hours only. Horbaczewski (quoted - loc. cit.) found that the hydrochlorate of pilocarpin administered to man in doses of 1/6 to 1/4 grain, caused an increase in the number of leucocytes + in the amount of uric acid eliminated. In the lower animals he found that- when administered hypodermically in doses of from 1/2 to 3 centigrammes per kilogramme of body-weight-, pilocarpin only produced an increase in the size of the spleen.

Black line = total leucocytes per cubic millimetre
Red line = polynuclear leucocytes per cubic millimetre
Blue line = mononuclear leucocytes per cubic millimetre
Violet-line = transitional leucocytes per cubic millimetre

Left-hand column indicates number of leucocytes.
Figures above = hours after injection.

Graphic Record after Pilocarpin Hydrochlorate (Exp't 2) and Carbolic Acid (Exp't 3)

Summary of Results of the Experiments

I The experiments show that none of the drugs used, affect the number of any particular variety of leucocyte; that is to say, one drug does not specially affect the numbers of one variety, & another the numbers of another variety: but the general plan of their effect is the same for all.

II In this general effect, the experiments not only agree amongst themselves, but also coincide with the numerous observations which have been made on the introduction of bacteria, bacterial products, & other foreign substances into the blood; the latter have already been mentioned in more detail in the earlier part of this thesis.

III The general effect of the drug-injections was to cause (1) a diminution in the number of the circulating leucocytes, with relative increase of the mononuclear variety (and "transitional"); followed by (2) an increase in the number of leucocytes, this increase being especially due to the relatively great addition of polynuclear forms.

IV There is evidently considerable difference amongst the drugs themselves, in the degree & duration of the 2 stages. The diminution stage was especially well-marked, & of comparatively long duration, after the injection of quinine hydrochlorate, atropine sulphate, Salicine, & Salicylate of Sodium:

and in these cases, the succeeding leucocytosis was relatively only slightly marked. On the other hand the injection of potassium iodide, antipyrin, thyroid tabloids, & pilocarpin, was followed in each case by a very marked leucocytosis, with a relatively slight preliminary diminution. The other drugs used, caused both stages in a moderate degree.
As was noted in the Remarks under the different drugs, both stages were not observed after every experiment, but there can be little doubt that this was owing to the blood not having been examined at the proper moment. That both stages did occur, is shewn by at least one experiment with each drug: when the leucocytopenia is of short duration, as some of the experiments shew it to have been, it may be easily overlooked. When the diminution of the total number of leucocytes is slight in degree, confirmation of its occurrence may be obtained by observing that the percentage of the mononuclear cells is increased, at the time of the counting.

V The percentage of the large mononucleated "transitional" cells does not vary so definitely as does that of the small mononuclear, but it appears to change in the same direction as a ~~whole~~ rule.

VI The large coarsely-granular eosinophile cells were not specially counted, but the examination of the stained films gives me the impression that they are diminished in number, relatively to the total

number of leucocytes, during the stage of hyperleucocytosis though not to the same extent as Sherrington found during the leucocytosis of acute local inflammation.

VII The changes in the number of leucocytes are to be explained, presumably, in the same way as the corresponding changes after the injection of bacteria etc. It is of some interest to note that two of the drugs which cause a marked degree of leucocytopenia, viz. quinine & atropine, have been observed by Horbaczewski (quoted by Hackbutton-Gulstonian Lectures-Brit. Med. Jour. 1893 Vol I p. 575) to cause an "atrophic" condition of the leucocytes: whilst after pilocarpin (which causes a specially marked increase) the leucocytes were active & undergoing karyokinetic changes.

VIII In some of the experiments, a fall in the number of the leucocytes was observed after the leucocytosis had passed off, to below the number counted before the injection was made.

The Action of Pilocarpin on the Structure of the Leucocytes.

As before noted, Expt. 7 of the Atropine series; and Expts 3 & 4 of the Pilocarpin series, were performed for the purpose of observing if any change took place in the structure or staining reactions of the leucocytes, under the prolonged administration of these drugs.
Both drugs are well known to have a powerful action upon certain ~~et~~ glands, and it was thought that these observations might throw some light upon the supposed possession of "glandular" secreting functions by the granular leucocytes. Some observations bearing on this question have been referred to in an early part of this thesis, when speaking of the functions of the leucocyte.
With regard to the effect of Atropine, although there was evidence of some changes in the leucocytes examined at different times during the course of the experiment, these were not of a sufficiently definite & constant character to enable any general conclusions to be drawn from them. I shall therefore not enter into a description of them here.

Action of Pilocarpin. Method of Observation.
At the various times when the leucocytes were counted (see tables of Expts 2 & 3), several films of the blood were obtained at each time. The films varied

somewhat from day to day, but usually films were taken shortly before & shortly after each injection, as well as at later periods after the injections.
The films were dried in the air, hardened by immersion for 2 hours in equal parts of absolute alcohol & ether, and then stained in various ways. Some were stained ~~by~~ with glycerine eosine, & haematoxylin; others with acid fuchsin & methylene blue: others again by the action of a warm saturated alcoholic solution of eosin for 1/2 hour; after which they were washed & dried, and then counterstained in strong aqueous solution of methylene blue for 15 minutes. The staining solutions used for all the films were of the same strength throughout; the duration of their action was carefully noted so as to be the same for all; and all the manipulations were performed with great care to ensure uniformity in the conditions of staining of each series of films. Finally, the stained films were mounted in Canada balsam.
The most satisfactory staining was obtained by the alcoholic eosine & methylene blue: but the structural appearances presented by the specimens of blood taken at any particular time were found to correspond closely in almost every detail, in films stained by all the three methods. This is important, inasmuch as if the changes observed had been due to differences in the strength & duration of action of the stains, specimens stained by other methods would not have shewn them.

A reliable method of testing whether a difference in the staining reaction of the protoplasm of leucocytes in two specimens of blood is really due to a difference in the cells themselves, or to a difference in the methods of preparation, consists in comparing the effect of the stain on the red corpuscles in the two specimens. If the red cells in both are stained of the same intensity, a difference in the intensity of the staining of the leucocytes must be due to a difference in the leucocytes themselves. Several dozens of films from the two experiments were examined, and the following is a ~~summary~~ short account of the changes observed in the leucocytes. No change was noticed in the appearance or staining reactions of the red corpuscles.

In the normal blood, before the commencement of the experiments, the nuclei of the small mononuclear cells stained deeply, & those of the large mononuclear to a rather less extent. The finely granular ~~eosinof~~ poly-nuclear cells were crowded with very distinct granules which took up the eosin (especially from the alcoholic solution) very readily: the nuclei were deeply stained by the methylene blue (or hematoxylin). These characters are well shewn in the accompanying drawing (Fig. 1.) The basophiles, & large eosinophiles with coarse granules, were also well stained.

So soon as 15 minutes after the first injection of 2 grains of Pilocarpin (in Expt. 4) there was a distinctly smaller number of granules in the finely granular oxyphile cells.

This change was noticed also on the following day.
On the 3rd day, 15 minutes after the injection, the polynuclear leucocytes present a striking absence of granularity. No refrachle granules are visible at all, and the protoplasm, though fairly well stained, is almost, if not quite, homogeneous. These features are shewn in the drawing (Fig. 2.)
15 minutes later, a specimen of the blood shews a few scattered granules in the leucocytes.
On the 4th day, in all the specimens, the leucocytes shew some sparse granules, which, however, are fairly stained. This applies to basophile, as well as oxyphile leucocytes.
On this day was first noted a change in the staining reaction of the nuclei, which now take up the blue stain badly. These characters are shewn in Fig. 3.
The sparseness of the granules in the oxyphile cells was observed every day after this, to the close of the experiment (Expt. 4): and in addition there was a further diminution in the degree of staining of the protoplasm & granules of these cells. Frequently the protoplasm seemed to be quite homogeneous & non-granular.
Further, the nuclei of the oxyphile cells also lost their power of staining deeply with the methylene blue: though this was not so constant a feature as the change in the cell-body. Fig. 4, drawn from a specimen on the 9th day of Expt. 4, and 15 minutes after the injection of that day, shews well the feebleness of the staining of the nuclei & protoplasm, with the absence of

of granularity of the latter. The red corpuscles are rather more feebly coloured in the figure than they appear in the specimen: in the latter they shew little or no difference from the normal.

It should have been stated before that the above description applies especially to the specimens of blood taken from the rabbit of Expt. 4, in which the daily injections were continued for 14 days: but the blood of Expt. 3 shews an exactly similar series of changes. A curious feature with regard to the large coarsely granular eosinophile cells was noticed, & is worthy of record. Several of these cells were found in two or three of the films in which the smaller oxyphile cells shewed the most marked absence of granularity & feebleness of staining reaction; and the former, in striking contrast with the latter, contained numerous well-stained granules: in fact they were exactly like those of the normal blood. It would seem therefore, that pilocarpin has no action on the protoplasmic granules of the coarsely granular cells, which are especially supposed to exercise a secreting action. On the other hand it causes, as already described, profound changes in the smaller oxyphile cells.

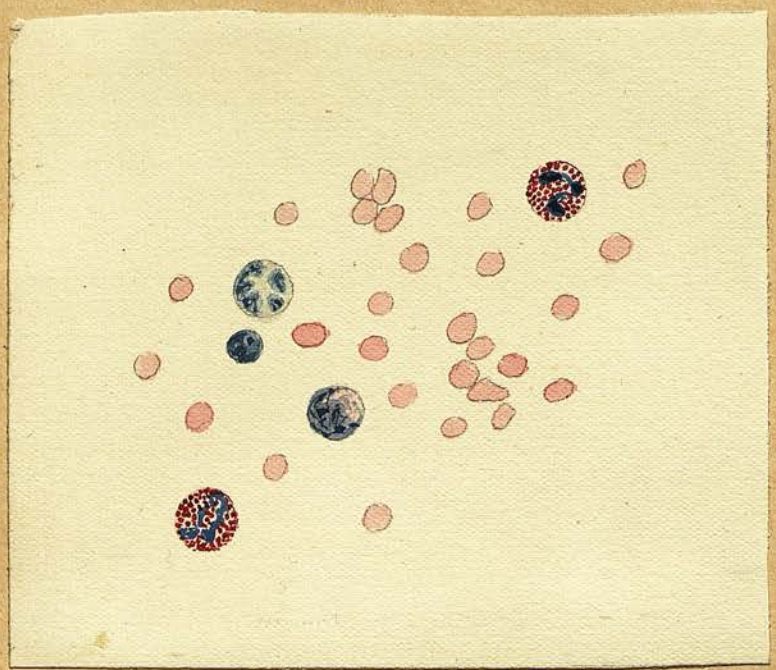

Fig. 1.

Blood of normal Rabbit- before injections of Pilocarpine: March 8. 1896

1/12 in. (oil immersion) objective: Zeiss' compensation eye-piece No 6.

The finely granular oxyphile cells are very characteristic.
The drawing also shows a small mononuclear cell, a large mononuclear, & a basophile.

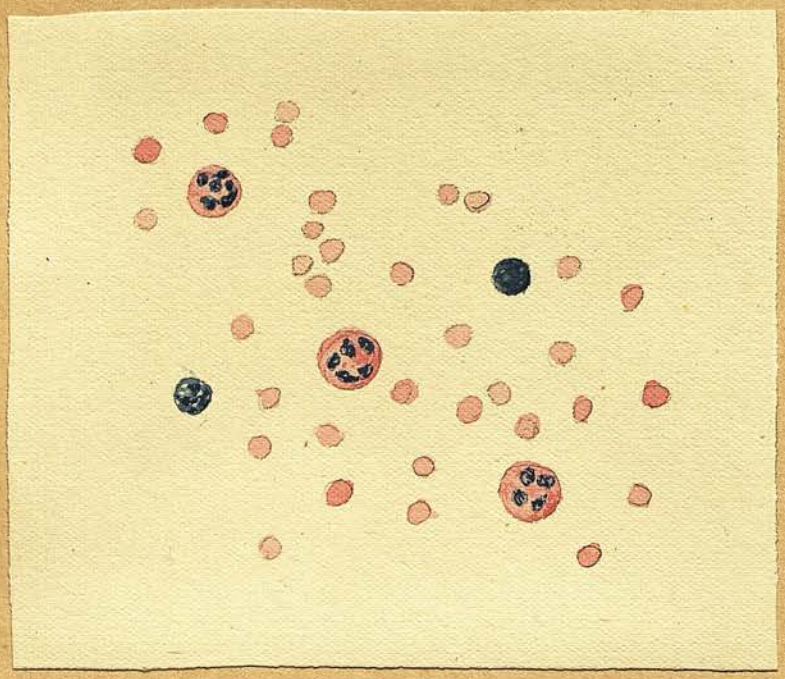

Fig. 2.

Blood 15 minutes after the injection of March 10th.

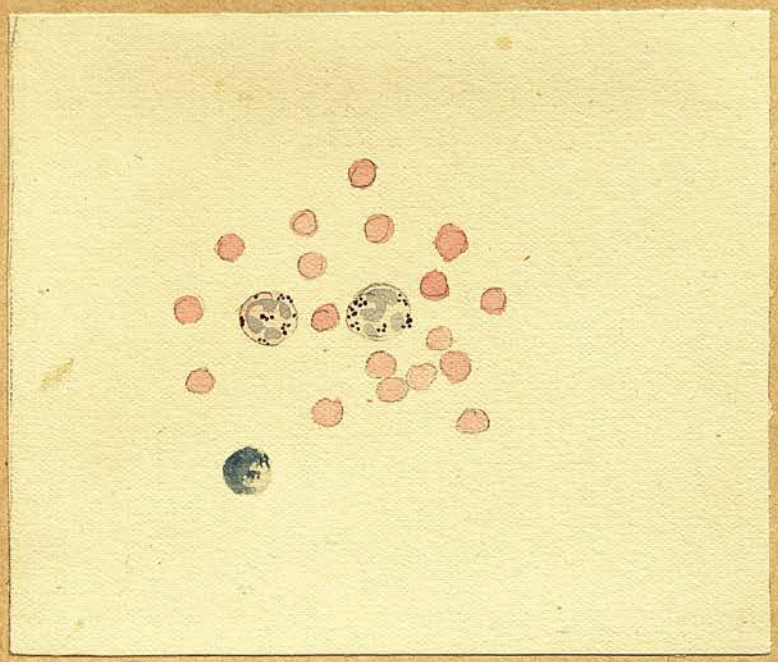

Fig. 3.

Blood. Calf an hour after the injection of March 11th.

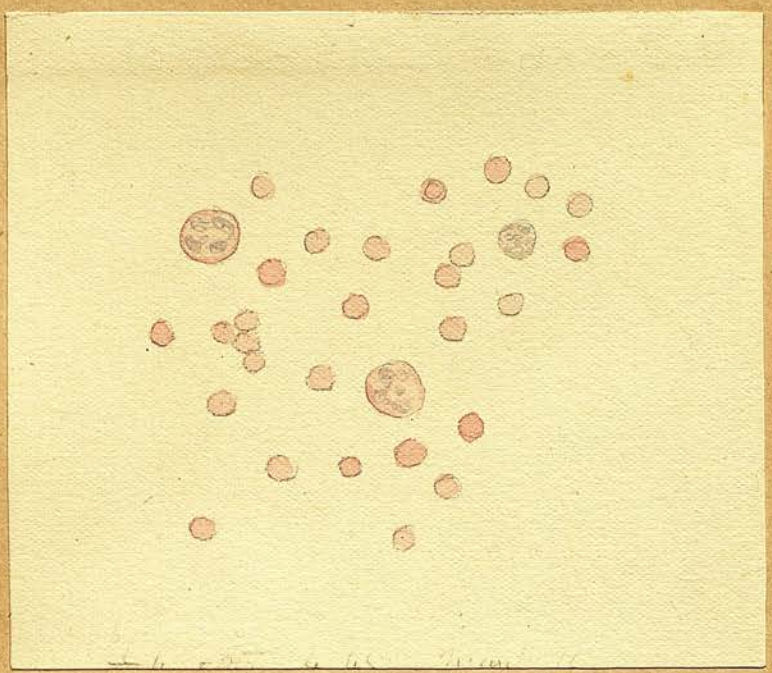

Fig. 4.

Blood 15 minutes after the injection of March 16th.